NATURE

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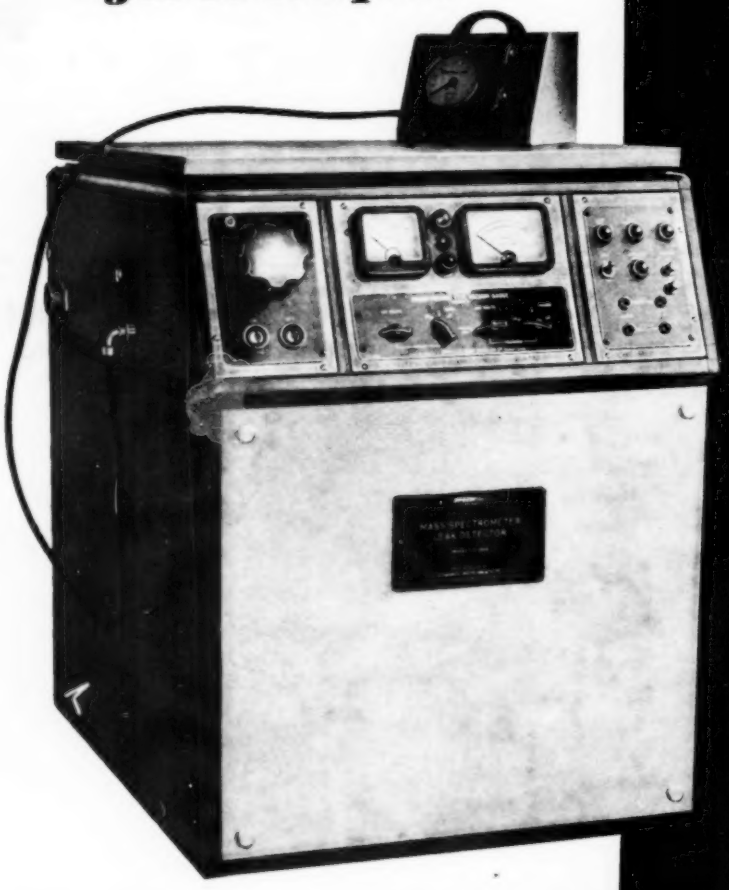

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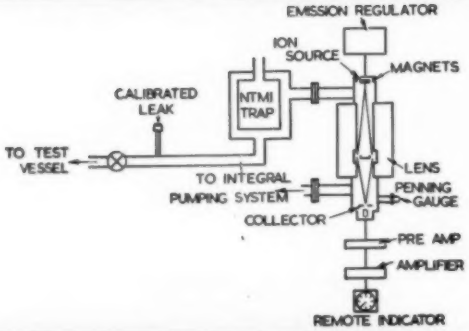

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THE BALANCE OF BRITISH SCIENTIFIC EFFORT

THE thirteenth annual report of the Advisory Council on Scientific Policy, covering the year from July 1, 1959, to June 30, 1960, and the first to be submitted to Lord Hailsham as Minister for Science, is concerned with one main topic: the balance of scientific effort. There is also a brief but important discussion of the implications for scientific education of the Crowther Report; and a paragraph on space research states that, after reconsidering the subject, the Advisory Council reaffirms its previous advice that a programme of scientific research in space using satellites on a purely national basis would not, in itself, justify the very large expenditure necessary for a full-scale programme, including the development of a launching vehicle. This in itself is a particular aspect of the balance of our scientific effort, and that comment applies equally to the section of the report which contains the Council's observations on two particular fields of pure science, optical astronomy and seismology, which have become, for various reasons, neglected. In addition to the detailed results of the review of the Statistics Committee on Expenditure on research and development in the United Kingdom, 1958–59, there are appended, as usual, details of the numbers of graduate teachers of science and mathematics in schools and technical colleges, degrees and diplomas in science and technology, and estimated Government expenditure on civil research.

The review of the balance of our scientific effort follows the two earlier general surveys undertaken by the Council described in its reports for 1953–54 and 1956–57. In the first of these, the Council expressed the view that "a country's wealth and power are to-day largely determined by the extent of its scientific knowledge and its capacity to use that knowledge". In the second the Council found that the resources devoted to research and development for civil purposes were still far too small for Britain's needs. Government expenditure on civil research was then estimated at some £26·6 million. Excluding Treasury grants-in-aid to the universities and expenditure on civil research by the Ministry of Aviation and the Atomic Energy Authority, expenditure has since risen from an estimated £30·45 million in 1958–59 to £38·868 million in 1960–61, and these figures should be compared with the estimate of £300 million for the expenditure by British manufacturing industry on research and development given in the Department of Scientific and Industrial Research's report, "Industrial Research and Development Expenditure, 1958", published last April (see *Nature*, 186, 997; 1960).

The present review was undertaken at the request of the Minister for Science, who asked the Council for an authoritative review of the balance of scientific effort in resources and man-power in the civil field, and in accepting this responsibility the Council has rightly attempted to define clearly both the aim and the limitations of any such review. It disclaims any attempt to seek a formal structure, but emphasizes rather the prime importance of devising criteria by which the points can be identified at which additional effort and resources will pay the greatest dividends. It is not concerned with administrative matters but with the strategy of scientific affairs, and suggests that it should be possible, on the basis of a comprehensive review, to indicate broad fields where further work is required, or where a new assessment or reorientation of effort seems desirable. It believes that the Research Councils and other government agencies operating in scientific and technological matters are the appropriate bodies to interpret its recommendations in terms of programes of work.

In its reference to strategy it does not mean to imply that the advance of scientific knowledge can be directed: it uses the term in the sense in which it has been used by Sir Henry Tizard and Sir Hugh Beaver, for example, and again in the Barlow Report on scientific staff. Basic advances in science depend on the creative individual, or perhaps also on the creative minority, but from time to time obstacles can be removed, encouragement and resources provided for people who have ideas and the best use made of our limited resources. In other words, the Advisory Council is seeking to act on the policy that has long influenced and guided such bodies as the Nuffield Foundation and Rockefeller Foundation, and identify the growing points and ensure that they are adequately supported.

Clearly an official body, even if its functions are purely advisory, cannot commit public support with the freedom with which the private foundation can allocate resources, but there is no more encouraging feature of the present report than the way in which the Minister for Science and the Advisory Council itself take such a wide view of the Council's advisory function. If this is sustained, Britain will have at last one body responsible for considering how new scientific developments may interact among themselves and with the functions of existing institutions. There is a welcome note both of confidence and of authority about the present report which promises well for the future, and suggests that the Minister intends to use the Advisory Council as it could and should be used.

In the present survey the Advisory Council had some general problems much in mind. If advance is to be made with economy of effort, the growing points of knowledge require review from time to time to ensure that there is maximum contact and inter-

action between different fields of activity, and that the greatest range of professional skill is brought to bear on them. Paying particular attention to the frontiers of traditional disciplines, the Council notes the need to organize studies which cut across customary educational patterns or organizations, in order to attack a central or particularly resistant problem. The growing points in science are most frequently found in areas lying between the traditional disciplines and often involve the participation of several of them. The rigid separation of these disciplines can be awkward; but the solution of such problems is not necessarily to be found in the creation of new university departments and new disciplines. The research institute offers one solution, though the Advisory Council believes that universities should consider carefully the patterns which are emerging and encourage the effective interaction of the traditional skills.

Undoubtedly the expansion of universities and perhaps especially the establishment of new universities offer opportunities for flexibility and experiment, not so much in the new universities, which have first to prove themselves, as in the older universities. The colleges of technology, too, could play an important part, and the Council thinks that current schemes for training increasing numbers of scientists and technologists should not be planned without due regard to the changes which are taking place and to the fields where further effort is required. It had repeated evidence that it is very necessary for educational and professional structure to keep in step with the rapid evolution of science and technology.

The Council appreciates that its survey has important implications for economic and financial policy which strictly fall outside its purview. Its recommendations concern both subjects selected for their intrinsic scientific value or potentialities, and others chosen because of their importance for the British economy, or the conservation of its natural resources. No survey in the field of technology can ignore economic factors, and the review demonstrates once again how scientific and technical factors penetrate into every field of public policy and require evaluation along with other factors if sound decisions are to be made.

The review is based on a number of separate inquiries designed to present the widest body of evidence and of informed opinion about current scientific effort. The statistical basis is provided by a comprehensive survey carried out by the Council's Statistical Committee and already noted as appended to the report. Information and advice were also sought from the four Research Councils as well as the Royal Society, the principal professional institutions and Government departments mainly concerned with important aspects of applied science or technology. A list of such departments, public corporations, learned societies and institutions which is appended, however, shows some curious gaps among professional institutions, the names of the Royal Institute of Chemistry, the Royal College of Surgeons and the Institute of Physics, for example, being absent, while it is also surprising that on the industrial side such bodies as the Federation of British Industries, the Association of British Chemical Manufacturers and the Society of Chemical Industry do not appear to have been consulted.

The result, nevertheless, appears to be a more comprehensive picture than was given in the Council's report for 1956–57, and the total expenditure of £480 million represents an increase in constant-value terms of about 40 per cent, or about 2·3 per cent of gross national product against 1·7 per cent in 1955–56. The pattern of cost of research in each sector, and to a lesser extent of sources of finance, remains broadly similar; but private industry is now financing 29 per cent of the total compared with 23 per cent previously, and the Government two-thirds instead of three-quarters. Non-defence expenditure has risen from £122 million to £243 million, and this expansion appears to be continuing. Estimates for the four Research Councils aggregate 37 per cent higher for 1960–61 than for 1958–59, but the table appended to the report shows a reduction in grants to learned and other scientific societies from £207,000 to £166,000 in 1960–61. Grants for overseas research increased from £1,735,000 in 1958–59 to £2,265,000 in 1960–61, and it should be noted that of a total Government expenditure on civil research of £30,453,000 in 1958–59 and £38,868,000 in 1960–61, £16,980,000 and £22,703,000, respectively, went to the Agricultural Research Council, the Medical Research Council, the Department of Scientific and Industrial Research and the Nature Conservancy.

Emphasizing that technological progress depends ultimately on the scientific work in the universities and technological institutions, the Advisory Council is once again concerned that British resources of highly qualified scientific man-power should be used as efficiently as possible in such institutions, and it believes that, to ensure this, further expenditure is required on improving equipment and facilities and in providing additional technicians. This may well be the more important with the further expansion of the universities and the establishment of new universities and colleges of technology, but it is also possible that the re-examination of teaching methods such as Prof. A. H. Cottrell and others have suggested could make a by no means negligible contribution to the efficient use of trained man-power. These are, of course, primarily matters for the universities and colleges themselves and for the professional institutions, and it is clear enough from the present report alone, and from what Lord Hailsham himself has said in the House of Lords, that the Advisory Council has no intention of endangering the autonomy of the universities. The present system of block grants for teaching and research through the University Grants Committee and of complementary earmarked grants for scientific research through the Research Councils appears to offer the minimum difficulties in administration and maximum freedom for the

universities; and the arrangements described in the Council's previous report for the periodic review and transfer of financial responsibilities for established research projects from the Research Councils to the University Grants Committee should make the system more effective.

Before discussing the survey in detail, the Council compares the United Kingdom expenditure on research and development in 1958 of £478 million, or 2·35 per cent of the gross national product, with the £3,971 million, or 2·74 per cent of the gross national product, expended in the United States. Whereas, however, in the United Kingdom the public sector accounts for 34·7 per cent of the cost of the work done, industry 57·1 per cent and the universities 4·8 per cent, in the United States the corresponding figures are 15·4, 75·3, 7·5 per cent, respectively. On the other hand, in the United Kingdom the public sector provides 68·5 per cent of the finance, industry 28·5 per cent and the universities 0·3 per cent, the corresponding figures for the United States being 61·0, 36·4 and 1·8 per cent, respectively. While the United States is devoting a somewhat larger proportion of its resources to research and development, it is probable that in both countries about 1·25 per cent of the gross national product is devoted to civil research and development work, and in this connexion the Advisory Council stresses the importance of using any opportunity which reductions in defence research and development may afford to increase the civil research effort in industry and elsewhere. It concludes further that a comparable scientific effort, in relation to the gross national production, with that of competitors should be maintained in Britain.

In regard to pure science, the Advisory Council's survey of the balance of effort leads it to the view that in pure mathematics Britain is losing ground, and that here the universities should give more attention to the provision of facilities for co-operative work. Oceanography and taxonomy have been relatively neglected, and in the section of the report which discusses the position of astronomy and seismology in some detail, the Council comments on the way in which British optical astronomy has lost considerable ground, partly in consequence of a low output of suitably trained specialists. Further steps are needed to bring astronomy to the notice of young physicists as a possible career, and while contemporary British resources in theoretical astronomy are substantial enough for an effective and relatively inexpensive contribution to British achievement and prestige, the proper deployment of these resources, which are scattered over a number of universities, is hampered by the lack of essential computing facilities. The provision of such large and expensive facilities may involve questions of university independence, and these are matters in which the universities may well be expected to take the initiative in co-operation, as in fact the Universities of Manchester and Liverpool have already done.

The Advisory Council endorses the recommendation of the British National Committee for Astronomy that the first priority for British astronomical activity in the southern hemisphere should be an immediate development of the potential resources of the Radcliffe Observatory; but on radioastronomy it makes no comment on the Jodrell Bank position and is content to express the hope that the two techniques, which are not alternatives, but complementary in the exploration of space, will be developed concurrently. The British weakness in seismology is particularly stressed, and if Britain is to regain her position in seismological research and provide the minimum background for participation in any international scheme for the inspection and control of nuclear explosions, seismological centres must be developed at one or two universities. Specifically the Advisory Council recommends that the existing activity at the University of Cambridge be expanded to provide such a centre or unit, with appropriate equipment facilities for routine and specialized experimental and theoretical work and provision for university teaching, and that a similar centre be created in the University of Edinburgh.

These recommendations regarding astronomy and seismology arise out of the Council's special examination of these fields rather than out of the general survey. The latter, however, directed attention to some fields in medical science where further research effort is desirable, such as mental illness, dental disease and social and industrial aspects of medicine. Some of the less-popular technologies also offer fruitful opportunities, and particular reference is made to a serious deficiency over the whole field of civil engineering. Here the Council recognizes that educational developments offer a real opportunity for a serious examination of the possibility of greater co-operation in the use of facilities between the universities and colleges of technology and Government establishments, but it also points to a few major topics where further work is needed. The Council had much evidence that engineering prospects are intimately bound up with the economic production of new materials—conducting, semiconducting, insulating, magnetic and structural—and the improvement of existing ones, particularly materials capable of operating at much higher temperatures.

This is a field demanding much-improved collaboration between chemists, physicists, mathematicians and metallurgists both in universities and in industry, while research into energy sources and their utilization also involves difficult problems alike in co-operation and in assessing the balance of effort. The Council considers that research effort on the utilization of coal should be strengthened, particularly on the development of processes for the complete gasification of coal, and it has asked the Minister of Power and the University Grants Committee to consider ways of strengthening studies in coal technology at universities or colleges of technology. The need for further application in industry of refined techniques of calculation and automatic controls is also stressed, as are the needs for work on the measurement and suppression of noise, research into

the environmental conditions and other aspects of important livestock groups, the metabolic diseases of sheep and cattle, the storage of fruit, and research into industrial ceramics to provide management with control techniques of appropriate precision. Finally, it comments on the significant absence of basic research in the scientific assessment of such major problems as the conservation and long-term utilization of British water-supplies, the basis for policies of land utilization, and it urges that new measures may be required to deal with basic research relating to the conservation of our natural resources.

This is one of the points concerning which the Council recommends some change in the organization of research. While the survey indicates that the general pattern is in broad outline satisfactory, one important gap in responsibility is apparent. There is no clear general responsibility for the conservation of our natural resources, whether of minerals, water, land-use, biological resources or fisheries. The responsibilities of the Nature Conservancy, and of other Departments concerned, cover only limited aspects, and the Council believes that there may be justification for a Research Council with central responsibilities for ensuring that, over the whole, scientific knowledge and research are brought to bear on all these issues. The problem is being examined in detail by the Council's Biology and Allied Sciences Committee.

Another point relating to the organization of research arises out of the high cost of some research projects, and has already been noted in relation to both astronomy and seismology. The general point is frankly faced by the Council, and the report points out that the attempt to pursue certain types of scientific activity in many different universities can so disperse and dilute the British effort that in the end she achieves much less than could be expected from the total expenditure involved. This is quite apart from considerations of man-power, and even if the universities were fully supplied with technicians on the scale desirable to avoid waste of highly trained man-power to which the Advisory Council has previously directed attention, there is still the possibility that large installations of equipment, which must be serviced by many specialized technicians and which has a high obsolescence-rate, may distort the pattern of a university and lead to a situation in which the direction of its research may be determined not by the scientific stature of the men available but by equipment available.

The Advisory Council cites nuclear physics as an example of these dangers, and believes that less dispersal of the British effort in the early stages of its post-war development would have given a greater return for our expenditure in this field. The situation has since been recognized by the creation of the National Institute for Research in Nuclear Physics, and while the Council notes with satisfaction recent developments in the regionalization of electronic computers and proposals for joint operation of nuclear reactors, it suggests that extension of the idea of such national institutes may require some adjustments of our scientific organization. Optical and radio astronomy, as well as space research, are other fields in which it is necessary to decide on the level of expenditure of resources and man-power that Britain is prepared to operate, and how dispersal of effort and unnecessary duplication of costly research facilities are best avoided. As alternative to a central institute available to workers in universities and similar institutions, it is suggested that such activities should be confined to one or two universities, as in fact it proposes for seismology.

The Advisory Council does not attempt to decide between these two alternatives, but it sees no other possibility if Britain is to participate effectively in such fields of research. Clearly the problem is one that should be engaging the active attention of the University Grants Committee as well as of the Research Councils; and the Advisory Council itself could well examine further the forms of organization and control appropriate to national institutes of this kind. Moreover, it may be necessary to extend the principle to the international plane, for in certain fields—the report instances space research, nuclear physics and oceanography—the point is fast being reached, if indeed it has not already been passed, at which international co-operation provides the only appropriate way of continuing effective work in these fields without subordinating the entire scientific effort of the United Kingdom to a few major programmes.

Finally, the Advisory Council observes that the problem is equally serious in industry. Fragmentation of the British industrial research effort can be a source of weakness, and of waste both in terms of money and of scientific man-power. Some degree of duplication in research may be desirable, and is indeed inevitable in the British economy with its multiplicity of independent industrial units; but it is notable that industrial leaders have of late been stressing the importance of co-operation in the attack on major problems of interest and importance to several industries. The Advisory Council points out further that there are to-day problems and projects of such magnitude in some fields that even large firms operating individually may only multiply, to no real purpose, research development and design work aimed at the same technical objectives.

No specific proposals are made for collaborative arrangements. The Advisory Council is content to remark that such co-ordination of effort is not merely essential but also possible and entirely compatible with a free economy. Without it Britain cannot hope to compete successfully in some of the newer fields of endeavour with other countries which are larger and wealthier, or which operate a political system in which industrial effort is co-ordinated centrally.

It may suffice here to note that the Advisory Council fully recognizes the bearing of education on the scale and effectiveness of the research effort, and that it welcomes many of the recommendations of the Crowther Report as likely to lead to education better suited to meet the scientific and technological

needs of Britain in the years ahead. This cogently argued and well-balanced report can only add to the stature and influence of the Advisory Council. It demonstrates that, in the Advisory Council for Scientific Policy, Britain now has a body capable of taking a comprehensive, balanced and authoritative view of the country's scientific effort, and assessing wisely the contributory factors. Its review shows clearly that there are still too many fallow fields and that there are also some fields in which activities could well become too large and distort the general pattern. The Council is clearly aware of the conditions which favour and encourage creative activity, and is concerned to do all that is possible to foster such conditions and to avoid any measures of adjusting the scientific effort which are detrimental to creative work. None the less, it does not hesitate where necessary to challenge the universities as well as industry, professional institutions and Government departments to the fresh thinking that may be required to shape existing arrangements or even institutions to new needs, and it is to be hoped that this sensible and balanced report will be closely studied by scientists and technologists and administrators whether in industry, in Government departments and public corporations, in research institutes or in universities and colleges of technology.

HISTORY AND PHILOSOPHY OF SCIENCE

Critical Problems in the History of Science

Proceedings of the Institute for the History of Science at the University of Wisconsin, September 1-11, 1957. Edited by Marshall Clagett. Pp. xiv+555. (Madison, Wisconsin: University of Wisconsin Press, 1959.) 5 dollars.

THE conference reported in this volume is symptomatic of the great and accelerating attention given in the United States to the history and philosophy of science. The work of the earliest pioneers of these themes in that country, George Sarton, Chauncey Leake and Henry Sigerest, is bearing fruit in the establishment of many academic posts to which, alas, though not unnaturally, distinguished English exponents are being attracted as they observe through a second generation the lack of such posts in their own land. Sixteen papers besides invited commentaries were contributed to the conference.

In the opening paper, Prof. Rupert Hall, discussing the respective shares in the Scientific Revolution of the scholar and the craftsman, points out that during the seventeenth century the "medieval association of scientific activity with the universities was weakened though not disrupted" not only by the importance in science of men who were not academically trained but also by the shift of scientific activity from universities to the scientific societies and to circles basking in exalted patronage. Further interesting contributions on this theme are by Profs. Robert Merton and Francis Johnson, Dr. A. C. Crombie and Father J. T. Clark.

In a stimulating contribution on the "Role of Art in the Scientific Renaissance", Prof. Giorgio de Santillana shows how art and science joined in the crucially important theory of perspective evolved by the architect Brunelleschi (1377–1446). He notes that Brunelleschi was accepted by his peers as an intellectual, and in this context he shows the importance of the literary skill of Leon Battista Alberti (d. 1472).

Prof. E. J. Dijksterhuis gives a brilliant survey of the precursors of Newtonian mechanics. It is impossible to epitomize his amazingly clear account of the development of European mechanics before Newton, but this paper well repays careful study.

"Contra-Copernicus: a Critical Re-estimation of the Mathematical Planetary Theory of Ptolemy, Copernicus and Kepler", is by Prof. Derek Price. It is written with this writer's usual clarity and beautiful figures, with some abatement of the credit usually awarded to Copernicus.

A number of papers consider the influence on science of social and political developments in Europe. Prof. C. C. Gillispie has a learned discussion of the *Encyclopédie* and Jacobin philosophy. He considers that a certain arrogance on the part of French scientists was responsible for the movement to promote an unlearned "natural history" and for the forcible closure of the Paris Academy of Sciences. Dr. Pearce Williams finds some explanation of what he, following Prof. Guerlac, calls the anti-Newtonianism of the leaders of the French Revolution in the movement that he terms "Enlightened Stoicism". Reform was sought through moral regeneration. By 1792, Condorcet presented his scheme for universal education throughout France, based on a simplified science.

Prof. Thomas S. Kuhn considers the influence on the formulation of the doctrine of conservation of energy exercised by the discoveries of conservation in other kindred fields. Prof. I. B. Cohen gives a no less convincing and fascinating exposition of the discovery of the conservation of the electric charge. Prof. Carl B. Boyer emphasizes the importance of nineteenth century observation of chemical weight.

Turning to biology, Prof. J. W. Wilson suggests "Biology attains Maturity in the 19th century". Prof. J. C. Greene epitomizes the views and influence of Auguste Comte and Herbert Spencer. Prof. R. H. Shryock expresses the hope that biology has not yet reached maturity. Prof. Conway Zirkle maintains that there is no evidence that either Darwin or Wallace was influenced by Victorian political or economic *laissez-faire* or belief in the benefits of competition.

Prof. C. S. Smith describes "The Development of Ideas on the Structure of Metals". His paper, although technical, is beautifully lucid. He discusses the influence on metallurgy of the revival of interest in atomic theory. Dr. Marie Boas gives an interesting account of "Structure of Matter and Chemical Theory in the 17th and 18th centuries".

Into this galaxy of fascinating information and reflexion are inserted valuable papers recording pioneer experience in teaching the history and philosophy of science. Prof. Dorothy Stimson records the consistent success of such studies with the students at Goucher College; Prof. Henry Guerlac relates that engineering students at Cornell have been equally enthusiastic; Prof. Bernard Cohen gives a cheering historical sketch of the teaching of the history of

science at Harvard over a period of sixty-eight years; Dr. Boas follows with an account of her happy experience in giving courses open to all upper-class university students in an Arts and Science School.

Prof. Guerlac gives an eloquent and convincing exposition of the function of the study of the history and philosophy of science in promoting true education.

It should be added that exceptionally full bibliographies are attached to all the papers. Space permits no adequate account of the important material packed into this volume. Both the conference and the publication were sponsored financially by the National Science Foundation of the United States and by the Research Committee of the University of Wisconsin. DOROTHEA WALEY SINGER

ELECTRICAL CHARACTERISTICS OF THE EARTH'S STRATA

Overvoltage Research and Geophysical Applications
Edited by James R. Wait. (International Series of Monographs on Earth Sciences, Vol. 4.) Pp. viii + 158. (London and New York: Pergamon Press, 1959.) 55*s*. net.

MEASUREMENTS of the electrical characteristics of the Earth's strata indicate the existence of several interrelated phenomena, which have been discussed from time to time in the literature. One of the effects is the potential which can be observed between two non-polarizable electrodes immersed in a porous medium, after the interruption of an applied step function current. This induced potential has a considerable bearing on the transient and frequency response of the medium. However, the phenomenon has, with a few exceptions, received scant attention.

The monograph edited by J. R. Wait consists of a collection of papers on the various aspects of induced potential. Various hypotheses for the occurrence of the normal effect observable in all naturally occurring porous media are fully discussed, and results of experimental work designed to test these hypotheses are given.

The emphasis of the majority of the papers is given to the geophysical application of the abnormal overvoltage effect observed in a porous medium containing electrically conducting particles, that is, metallic ores.

Theories of the transient and frequency response characteristics of such media are suggested, and the results of both laboratory and field investigations are given and discussed in relation to the suggested theoretical treatments. A short but useful paper on the field equipment required for prospecting for metallic ore bodies by the overvoltage method is included.

Some of the hypotheses advanced and indeed some of the practical results reported do not altogether agree with other published data on the subject. These differences are, however, mainly in degree rather than in fundamental concept. There appears to be, for example, some discrepancy between the discussion of the variation of measured conductivity of porous media with frequency, when this is compared with earlier reports. This discrepancy may be real and, in fact, represent a difference in the response obtained from porous media with and without conducting particles.

The authors have given factual reports of the results of a concerted attack on the problem of the overvoltage phenomenon and its practical application to geophysical prospecting. The individual papers are well presented and the various discussions are logical and thought-provoking. The collection is well worth study by all research geophysicists, and should be read by anyone concerned with the flow of electrical current through Earth strata. P. THREADGOLD

NATURAL PRODUCTS

Konstitution und Vorkommen der organischen Pflanzenstoffe (exclusive Alkaloide)
Von Dr. Walter Karrer. (Lehrbücher und Monographien aus dem Gebiete der exakten Wissenschaften. Chemische Reihe, Band 12.) Pp. 1207. (Basel und Stuttgart: Birkhäuser Verlag, 1958.) 136 Swiss francs; 136 D.M.

NATURALLY occurring organic compounds have fascinated chemists for very many years. Their interest began with substances possessing some easily recognizable property, such as colour, smell, bitterness, or toxicity, but to-day it ranges through the whole spectrum of natural products. In spite of this interest and the current rapid developments in an understanding of the biogenesis of such compounds, there has been no up-to-date dictionary of natural products. Although monographs are available for some limited groups such as carbohydrates, alkaloids, steroids and carotenoids, in the case of many groups, or even individual compounds, the collection of comprehensive information has entailed a long and tedious literature search. Dr. Walter Karrer has now eased this difficulty to a considerable extent. He has collected together details of organic compounds of known, or apparently known, structure found in plants, other than alkaloids. Even with these limitations about 2,700 natural products are described, ranging from hydrocarbons through depsides, carotenoids and terpenes to nitriles, pyrimidines and nucleotides.

The structural formula of each compound is given, as well as details of its distribution in the plant world. Key references to the elucidation of structure and synthesis are also listed. The information given about those groups of compounds with which I am familiar is accurate and comprehensive, the literature being covered to 1956, and the author is to be congratulated on the immense task which he has successfully undertaken.

For those concerned with structural elucidation of natural products, with their pharmacological activity, or with the biosynthesis of organic compounds, the appearance of this volume emphasizes the need for an even more complete dictionary of all natural products. Many compounds have been isolated which are inadequately described in the literature or the structure of which is still unknown. Often the same compound is described under different names. If some chemist would collect together the information about these compounds with the same thoroughness that Dr. Karrer has shown in the present volume, he would perform an invaluable service.

T. G. HALSALL

MOLECULES IN CRYSTALS*

By Dr. DOROTHY HODGKIN, F.R.S.
Chemical Crystallography Laboratory, Oxford

ON this occasion, I wish to describe the structures, as we see them to-day, of a number of very remarkable crystals. These would certainly have interested the early Fellows of the Royal Society and particularly perhaps Hooke and Boyle, both of whom carried out experiments on growing crystals, observed the shapes of their faces and speculated on the arrangement of corpuscles within them. In his "Micrographia", in 1664, Hooke drew pictures of alum crystals side by side with sheets of globular bodies in hexagonal and cubic arrays (Fig. 1) and commented: "I think, had I time and opportunity, I could make probable, that all these regular Figures that are so conspicuously *various* and *curious* . . . arise onely from three or four several positions or postures of Globular particles, and those the most plain, obvious and necessary conjunctions of such figur'd particles that are possible . . . by these kinds of texture or position of globular bodies, may you find out all the variety of regular shapes into which the smooth surfaces of *Alum* are form'd . . . nor does it hold in superficies but in solidity also, for it's obvious that a fourth *Globule* laid upon the third in this texture, composes a regular Tetrahedron, which is a very usual Figure of the *Crystals* of *Alum*".

Boyle imagined the integral parts of a body might have a variety of shapes, not only globular. Among the Qualifications that conduce to Fixity, he imagined would be "the ineptitude of the component corpuscles for avolation by means of their branchedness, irregular figures, crookedness or other inconvenient shape, which intangles the particles among one another and makes them difficult to be extricated".

The structure of alum is considerably more complicated than Hooke's drawings propose. As we know it now, it is based on a series of studies made by Cork, Beevers and Lipson, using X-rays, and most recently, by Bacon and Gardner, using neutron diffraction. Each unit cell contains 4 aluminium ions, 4 potassium ions, 48 water molecules, 8 sulphate ions. Remarkably enough, the relative positions of the positive ions in the crystal conform with Hooke's suggestions. But the intermolecular attractive forces which hold the units together are clearly more complicated than simple close packing. Around each trivalent ion are 6 water molecules, in a regular octahedron, each exactly oriented with the hydrogen atoms directed symmetrically away from the metal ion: and the metal to oxygen distance here is relatively short—1·94 Å. Around the monovalent ions, the six water molecules have a more open, irregular arrangement with oxygen–potassium 2·97 Å.; and between the oxygen atoms of the water molecules and sulphate ions various short contacts can be traced, the shortest being those where one hydrogen atom lies in the direct line between the two oxygen atoms. The whole atomic

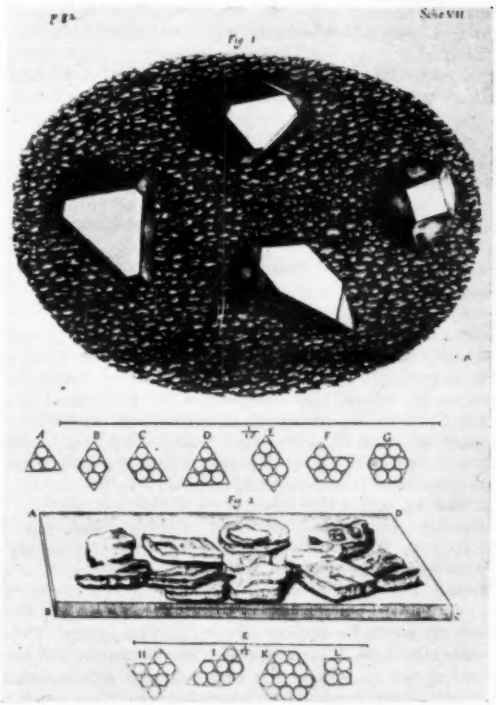

Fig. 1. Drawing from Hooke's "Micrographia" showing alum crystals and close-packed spheres. (*A*, *B*, *C*, *D*, Fig. 2 is an illustration to another section of the book)

arrangement is seen to result from a delicate balancing of a number of interlocking chemical and physical and geometrical requirements.

The knowledge we have of the exact arrangement of the atoms in this and many other crystals has been obtained as a direct result of the discovery of X-ray diffraction of crystals by von Laue, Friedrich and Knipping, and its development as a tool for structure analysis by W. H. and W. L. Bragg nearly fifty years ago. It is particularly sad that Prof. von Laue died only some months ago—but also a particular pleasure to think that he must have known before his death of the latest and most spectacular results of his discovery—results with which Sir Lawrence Bragg has been almost as intimately associated as he was in the first solution of any crystal structure.

The impression that I wish to give is one almost of direct sight of the atoms in crystals, an impression which has followed particularly from the use of Fourier series in interpreting the diffraction effects. In 1915, W. H. Bragg pointed out that the periodic

* Substance of a Royal Society Tercentenary Lecture delivered on July 23.

variation of the medium in crystals could be analysed by Fourier's method into a series of harmonic terms, the existence of which was indicated by the series of spectra obtained by diffraction from a given set of crystal planes. The recombination of the observed harmonic terms in a Fourier synthesis is now usually carried out mathematically to provide a physical measurement of whatever periodic structure is responsible for the observed diffraction effect—the electron density, if X-rays are used, potential distribution in electron diffraction, nuclear scattering power in neutron diffraction. The formulæ involved may be given in the form

$$\rho_{xyz} = \frac{1}{V}\sum_{-\infty}^{\infty}\sum_{-\infty}^{\infty}\sum_{-\infty}^{\infty} |F_{hkl}| \cos(\theta - \alpha_{hkl}) \qquad (1)$$

$$\text{where } \theta = 2\pi(hx + ky + lz)$$

$$F^2_{hkl} = [\sum_n f_n \cos\theta_n]^2 + [\sum_n f_n \sin\theta_n]^2 \qquad (2)$$

$$\tan\alpha_{hkl} = \frac{\sum_n f_n \sin\theta_n}{\sum_n f_n \cos\theta_n} \qquad (3)$$

Here the first equation shows that ρ, the electron density in the case of X-ray scattering, can be found at a position xyz in the crystal cell by summing a series of which the coefficients of the terms $|F_{hkl}|$ are given by the measured intensities of the diffracted spectra. Both these intensities and the phase-angles α associated with them depend on the actual positions of the atoms in the crystal, as shown in the equations 2 and 3; while the intensities of the spectra may be directly measured on photographic films or by counters, the determination of the phase-angles presents more serious problems. Usually they are found through a series of approximations to the exact atomic distribution often involving trial structures and a variety of calculations, many of them also based on Fourier series; for example, F^2 series and series in which the phase-angles are calculated on some parts only of the structure, often with the coefficients modified by the subtraction of certain calculated atomic contributions.

The X-ray analysis of the alums happens to occupy a rather special place in the history of the crystallographic use of Fourier methods. In their investigation, it was first pointed out that by the study of an isomorphous series of crystals the phases as well as the amplitudes could be experimentally determined, and a direct picture of the atomic arrangement formed by Fourier synthesis without preliminary trial calculations. In the alums, the scattering power of the atoms can be changed by chemical substitution, for example, aluminium may be replaced by chromium, potassium by ammonium, rubidium, cæsium or thallium, sulphur by selenium; and a direct calculation of the one-dimensional electron density distribution between two planes in alum crystals was first made by Cork in 1927. The crystallographic situation here is very simple since the alum crystal structure is centro-symmetrical. But the formal equations are clearly soluble for the three-dimensional asymmetric case, given three or more isomorphous crystals, as has been effectively demonstrated in the solution of the most complicated crystals so far analysed.

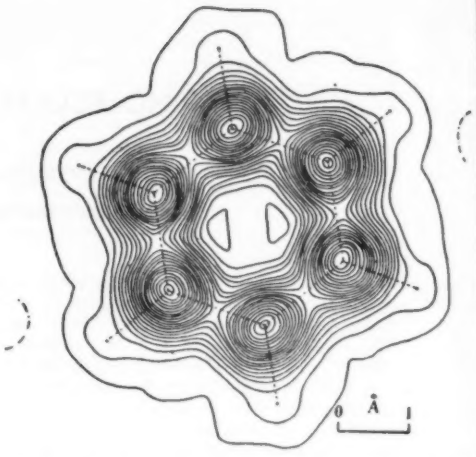

Fig. 2. Electron density in the plane of the benzene ring. Contour interval, 0·25e/Å.^{3} (from Cox, Cruikshank and Smith, *Proc. Roy. Soc.*, A, **247**, 1; 1958)

It has become common in the past few years with the advent of electronic computers to calculate the electron density in crystals in three dimensions. With simple crystals, these calculations can provide a highly exact picture both of the positions and of the modes of vibration of the atoms in the crystal. The analysis of benzene at −3° by Cox, Cruikshank and Smith may be quoted as an example. The electron density in the plane of the benzene ring (Fig. 2) shows rather distorted peaks over the carbon atoms. If the density to be expected from spherical carbon atoms is subtracted from this pattern, we get difference peaks which show the positions of the hydrogen atoms and also indicate atomic movements—that the molecule, as a whole, is oscillating with a root mean square amplitude of approximately 7·9° about the six-fold axis. Such a large amplitude of oscillation is permitted by the non-directed character of the intermolecular forces and cog-wheel packing of the molecules in the crystal. In diketopiperazine crystals the situation is rather different. The molecules are hydrogen-bonded together in bands in the crystal; the atomic positions are very accurately defined to within 0·004 Å. through the X-ray analyses of Corey, Degeill and Marsh and electron diffraction studies by Vainstein. In the latest electron density maps calculated with data obtained with molybdenum $K\alpha$ X-radiation, the atomic maxima appear very sharp (Fig. 3). The contour interval is 8 times that on the benzene map, and the thermal parameters derived for each atom have been analysed by Dame Kathleen Lonsdale. It appears that in this crystal the chains of molecules linked by hydrogen bonds are behaving like bundles of sinuous and somewhat elastic ribbons. The easy movements are those by which the atoms move out of the plane of the ribbon, either by a translational or librational movement of the molecule as a whole. Translation of the molecule within the chain is slightly more difficult, and there seems very little oscillation about the normal to the ring system: by contrast with benzene, the root mean square amplitude of oscillation is here only 1·7°.

Once allowance has been made for thermal motion in interpreting electron-density maps, the deviations of the peaks from spherical symmetry seem extremely

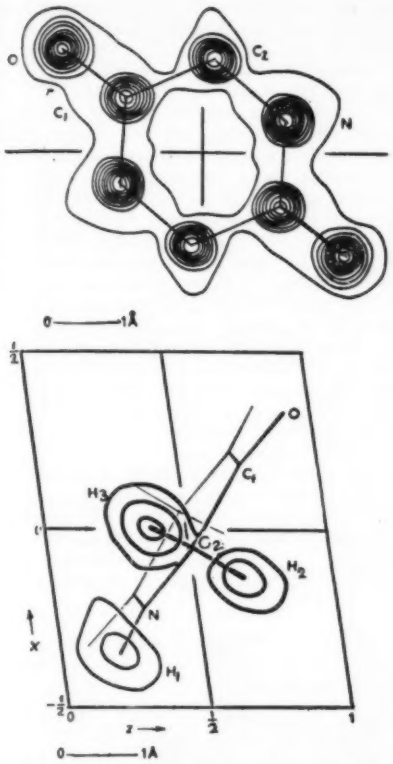

Fig. 3. Above, electron density in the plane of the ring in diketopiperazine. The contour interval is $2e/Å.^3$ beginning with the 1-electron contour. Below, a composite drawing of the electron density over the hydrogen atoms. The contours are at intervals of $0{\cdot}25e/Å.^3$ (from Degeill and Marsh, *Acta Cryst.*, **12**, 1007; 1959)

small: more accurate work at varying temperatures is required before these deviations can be taken very seriously as evidence of different varieties of chemical linking. Still, at the level of good approximate structure analysis, many interesting observations have been made. Some of these illustrate the ways in which molecules are restricted in their atomic arrangement, the flatness of aromatic or multiple-bonded systems, the staggered conformation of carbon chains, the tendency to form hydrogen bonds in certain preferred directions in relation with hydroxyl, amino and carbonyl groups. In oleic acid crystals, for example, Abrahamsson and Nahringbauer find long V-shaped molecules, with planar double bonds and staggered chains, associated through carboxyl groups. In *i*-erythritol—analysed by Powell and Bekoe—small compact molecules, with hydroxyl groups on each of four carbon atoms, are knit together by two systems of hydrogen bonds in a complex tetragonal arrangement. In hexagonal crystals, the molecules of the amino-acid, cystine, have a spiral arrangement about a six-fold screw axis—identity requires a lattice repeat interval of 56 Å. There are close hydrogen-bonded contacts between amino and carboxyl groups within one layer, the layers being linked by S–S bridges. Observations on crystals such as these provide a solid basis of facts in the light of which we can interpret data from more complex molecules.

The electron-density patterns obtained from these crystals are mostly derived by a series of approximations based on trial structures. These approximations are easier to interpret if one or two atoms in the molecule are relatively considerably heavier than the others. The heavy atom positions can then usually be found unambiguously and their scattering contributions used to initiate a series of approximate electron density calculations. The effects have been exploited in a large number of recent analyses aimed at the determination of the actual chemical structure of natural products, sterols, terpenes and alkaloids, a variety of molecules with fantastic names and fantastic condensed ring systems, calycanthine, limonin, *iso*clovene, mircestrol—many others. Limonin, examined by J. M. Robertson and his students very recently, was effectively analysed twice over in the same crystal, since the derivative used, epilimonol iodoacetate, crystallizes with two non-crystallographically identical molecules per asymmetric unit. The character of the analysis may be illustrated by a half-way stage such as the third calculated three-dimensional electron density distribution (Fig. 4).

Here heavy peaks indicate atomic positions recognized earlier and used in calculating phase angles, small ones show newly appearing atomic sites—the relatively unknown are differently distributed in the two molecules. Both the detailed molecular structure revealed and the fitting of the molecules in the crystal make one astonished at the variety of complications present—differently sized and shaped ring systems and side-chains. An earlier example of a similar analysis—which grew out of Bernal's first attack on the sterol structures—is that of calciferol which, in the form of the iodonitrobenzoate, also has a very intricate crystal structure. Both these illustrate remarkably well the difficulties of crystal growing and melting which Boyle envisaged in relation to the "branchedness" of the building units.

Considered simply as an aid to X-ray analysis, heavy atoms seem to work best at a level at which

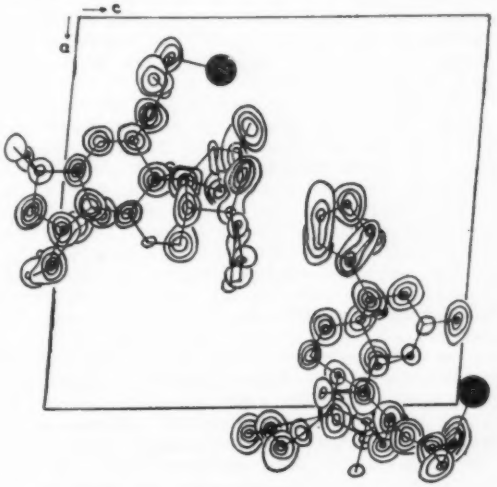

Fig. 4. Electron density contours taken from the third three-dimensional distribution calculated for epilimonol iodoacetate (after Arnott, Davie, Robertson, Sim and Watson, *Experientia*, **16**, 51; 1960)

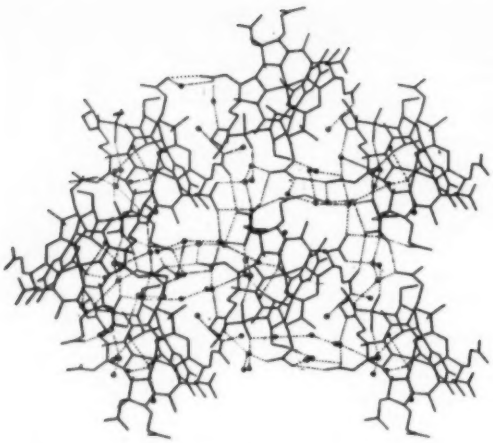

Fig. 5. Atomic positions in wet vitamin B_{12} crystals projected along *a*. The molecules in one layer are shown, together with a single molecule and the phosphate groups of molecules in the second layer. Small circles mark probable water molecule positions; dotted lines mark hydrogen bonds

their scattering power is roughly equal to that of all the other atoms present—for example, one bromine atom to about 24 carbon atoms, one iodine atom to 56 carbon atoms and so on—remembering that the scattering contribution is roughly proportional to the square of the atomic number. But much smaller relative differences can be exploited, as in the determination of the structure of vitamin B_{12}, where the heaviest atom, cobalt, contributes only one-tenth of the total scattering effect. Here the finding even of the exact molecular formula, $C_{63}H_{88}O_{14}N_{14}P\,Co$, depended on the gradual sorting of the atomic positions from various approximate electron density maps until it became clear which atoms belonged to the vitamin molecule itself, which to the surrounding water of crystallization. The knowledge from parallel chemical research that a benziminazole, sugar and phosphate group were parts of the molecule, greatly assisted the sorting process. But their relative positions in the molecule and the whole of the structure of the porphyrin-like nucleus followed from the X-ray analysis alone.

The vitamin B_{12} molecule proves to be quite compact, organized around the two planes of the inner porphyrin-like nucleus and the benziminazole group. Its geometrical form can be illustrated by a calculated projection of all of the atomic positions on the plane passing through the cobalt atom and four inner nitrogen atoms of the nucleus. From this projection it is clear that the benziminazole ring lies across the nucleus in a position mainly determined by packing considerations; it is also tilted about 10° from the direct normal to the projection plane and the nucleus is a little buckled where the two rings make contact. The different amide side-chains lead off from the nucleus in generally staggered arrangements; small deviations from these appear to be due to conflict with the requirements of intermolecular contacts, particularly through hydrogen bonds.

In outline the crystal structure is simple (Fig. 5). The roughly spherical molecules are arranged in approximately hexagonally close-packed layers, the two layers in each crystal unit being slid a little distance from the formal positions required by close-packing, with the spaces between the layers occupied by water molecules. But in detail, the atomic arrangement is very intricate, a consequence of the exact molecular geometry. In wet B_{12}, where the crystals are suspended in their mother liquor, the acetamide and propionamide groups all make direct intermolecular hydrogen-bonded contacts—the B_{12} molecules are in contact with one another on every side. Between them, the water molecules, approximately 22 per B_{12} molecule, make additional hydrogen bonds with the active groups, particularly with the amide and phosphate groups and with one another. Along particular lines in the crystal structure, water is continuous from one side of the crystal to another and is probably trickling through it during X-ray photography in narrow streams, one molecule deep. As Sir Francis Simon suspected when he examined a model of the structure, the atomic arrangement is not the same in every unit cell throughout the crystal and, as the crystals are picked out of their mother liquor, a somewhat irregular transformation occurs. A few water molecules leave every unit cell, the B_{12} molecules slightly change their relative positions, two amide groups swing round carbon–carbon bonds and check the flow of water and there results a new, more imperfect but still largely crystalline molecular arrangement. The electron density calculated throughout the water molecule region in the air-

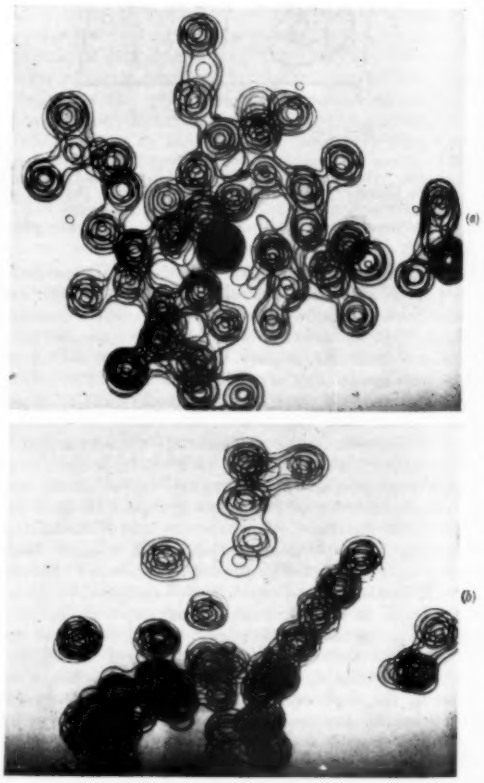

Fig. 6. Superimposed sheets showing the electron density calculated over the molecule in wet vitamin B_{12} crystals

a, The corrin nucleus and its surroundings; *b*, the benziminazole nucleus, seen edge on, and its surroundings

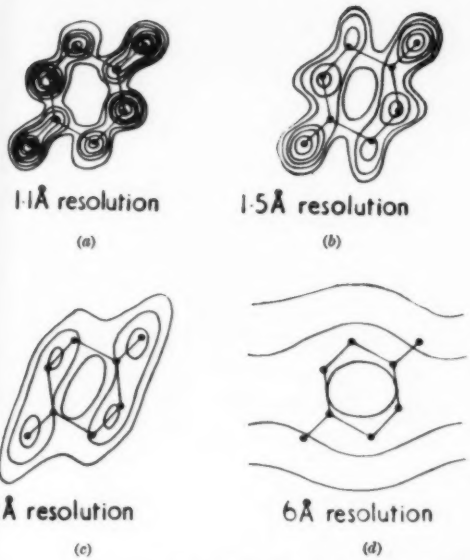

Fig. 7. Electron density in diketopiperazine calculated with reflexions limited at different spacings

dried crystals illustrates different degrees of order within the crystal pattern. Some water molecules, such as those hydrogen bonded to the phosphate oxygen atoms, are represented by high peaks; others by low ridges which seem to represent no more than the regions in which moving water molecules have been stranded in different unit cells as the crystals dry.

The atomic positions in the electron density maps for B_{12}, though distinct, are not so well defined as those shown earlier—a consequence of the smaller number of observable X-ray reflexions (spacing limit, 1·1 Å.) which in turn may be due to irregularities in the crystal introduced by the moving water molecules (Fig. 6). As the spacing limit is still further reduced the electron density pattern becomes increasingly blurred—the appearance of diketopiperazine in Fig. 7, calculated with different numbers of X-ray reflexions, shows the effect very clearly. The first drawing corresponds with the limit of reflexions observed for B_{12}, the second and third to limits of 1·5 and 2·0 Å. respectively, the fourth to 6 Å. At the fourth all individual atomic definition has disappeared. But at the third, although the pattern is much blurred, it is easy to recognize the molecule provided one knows approximately what to look for.

Diketopiperazine as an example of atomic definition, and these particular cut-off limitations on our view of atomic positions, have been chosen to illustrate our evidence on the structure of the protein crystals, myoglobin and hæmoglobin. The molecules here are twelve to fifty times as large as B_{12} and composed of amino-acid residues linked in peptide chains. Many earlier lines of evidence indicated that within these molecules the peptide chain must be composed into a structure that had itself something of the character of a crystal lattice. Lindeström-Lang described in imagination a protein molecule as itself a small crystal, in equilibrium with the surrounding medium, with the peptide chains within it knitted together by forces well known from macrocrystals—van der Waals' forces and hydrogen bonds. It was Astbury who recognized that X-ray diffraction effects from many proteins suggested the chains were present in a specifically folded form, the α-fold for which Pauling, Branson and Corey proposed in 1951 a particular chain configuration, the α helix, following the long series of exact peptide structure X-ray analyses which began with diketopiperazine. To-day it is possible to see within myoglobin crystals the peptide chain and its configuration—indeed to visualize the positions of some 1,200 atoms as well as the 8 which occur in Fig. 7*c*; while in hæmoglobin the peptide chain is as clearly outlined as the molecule as a whole is defined in Fig. 7*d*. This result has been reached through the very remarkable series of researches of Perutz, Kendrew, and their collaborators at Cambridge, who have exploited certain features of protein crystals to make a whole series of accurately isomorphous crystal structures.

Protein crystals were early observed occurring naturally in plant and animal tissues; one of the first described in the literature was the hæmoglobin of the earthworm (Hünefeld 1840), which crystallizes spontaneously in blood left to dry on slides. Early too, in the middle of the nineteenth century, one of the important properties of these crystals was recognized by Schimper—that they contain large volumes of water of crystallization penetrable by ions and by quite large molecules such as organic dyes without apparent disturbance of the crystal lattice. This property can be used to introduce heavy atoms into the crystal in order to make isomorphous derivatives of the proteins for X-ray analysis. The first such heavy atom isomorphously introduced, mercury in sodium *p*-chloromercury benzoate, was chemically attached by the SH groups in hæmoglobin: others have been found to enter and lodge in definite positions in the crystal lattice by crystallizing the proteins in the presence of heavy atom-containing ions or molecules and by soaking them in appropriately buffered dilute solutions of the reagents. The molecules which have been shown to lodge in hæmoglobin and myoglobin are a varied collection—*p*-chloromercury benzene sulphonic acid, mercuridiammine and aurichloride derivatives, *p*-iodonitrosobenzene, which reacts with the iron atom in the hæm group of myoglobin, among others. Measurements of the intensities of some 10,000 X-ray reflexions from each of five different myoglobin derivatives and of several hundred reflexions for six different hæmoglobin crystals have been made; and with the F_{hkl} values derived for these the full potentialities of equations (1) (2) and (3) have been exploited to derive phase angles and calculate directly in three dimensions the electron density in crystals of sperm whale myoglobin and horse methæmoglobin.

So far, two three-dimensional electron density distributions have been calculated for the sperm-whale myoglobin crystals. The first of these, at 6 Å. resolution, shows the main back-bone structure of the molecule, a series of rod-shaped units linked together by strands of density and surrounding a rather heavy maximum, clearly the iron atom of the hæm group. At 2 Å. resolution the rods and strands break up and, to an astonishing degree, the calculated density pattern defines the whole of the atomic distribution in myoglobin (Fig. 8*a*). One can see,

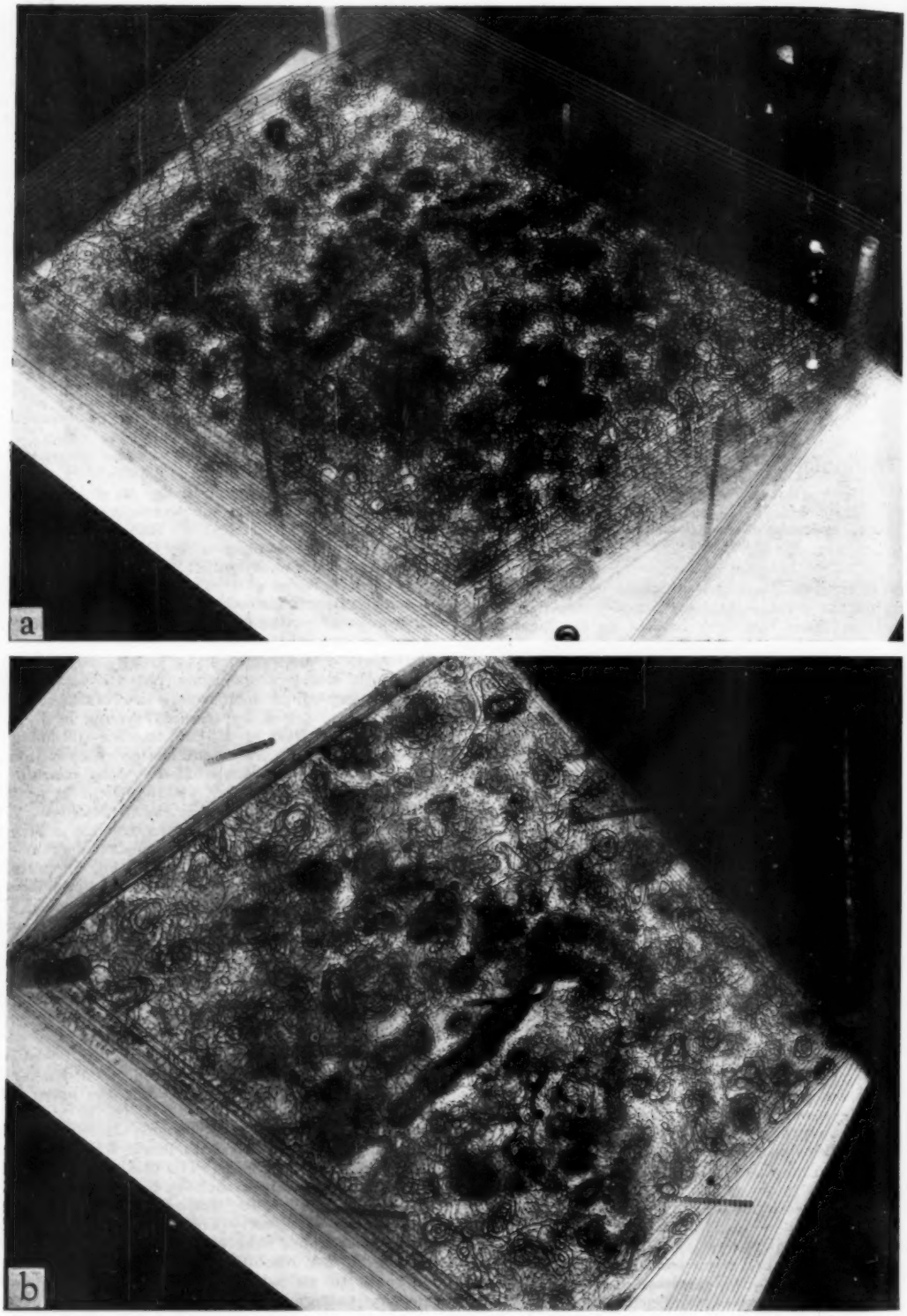

Fig. 8. Electron density calculated at 2 Å. resolution for myoglobin. The contour intervals are at 10·32e/Å.^{3} with the first contour dotted, the electron density contours being drawn on sheets stacked together. *a*, To the right, one is looking down an α-helix, clearly marked by the concentration of density around a hole of low density; *b*, the model tilted to show the hæm group on edge

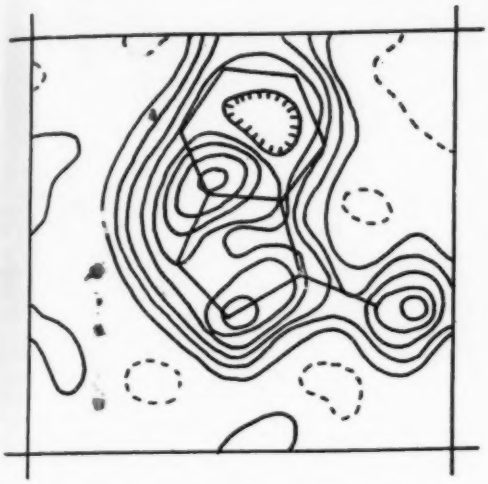

Fig. 9. Electron density contours defining a tryptophan residue in myoglobin. Contours at 1/5e/Å.³

within each rod-shaped unit, the peptide chain wound in precisely the configuration required in a right-handed α-helix; there are in all eight distinct stretches of α-helix of very varying numbers of residues—one 24, one 19, one 18, two 16, one 8, two 7 residues long. These lie generally at considerable angles to one another and are linked either directly or by small stretches of open chain—one, two, three or more residues long. Projecting from the main chains are the different amino-acid side-chains and these too are surprisingly recognizable—the very flat aromatic units, phenylalanine and tryptophan (Fig. 9), for example, are easily distinguished from branched valine or extended staggered leucine; a terminal atom turning back towards the peptide backbone or to an isolated peak—a water molecule—as if to form a hydrogen bond helps to identify hydroxyl groups as in serine or threonine. Nearly the whole of the sequence of 153 amino-acid residue in the molecule can be traced—and much of it has already been checked with parallel chemical studies of Edmondson. The hæm group itself is very clear (Fig. 8*b*). On one side of it there is a single peak, probably a water molecule, attached to the iron atom in the same way as the water molecules to the chromium or aluminium ion in the alums. On the other side is a histidine group, corresponding with the benziminazole group in vitamin B_{12}—and like it arranged with its plane at a general angle, about 10°, to the nearest Fe–N bond.

There are main chains—as suspected earlier—lying on either side of the hæm group and from these project other residues, mainly aromatic, tyrosine, phenylalanine, proline, and one other histidine residue. The molecules make contact with one another on every side in the crystal, directly and through water molecule bridges; they enclose channels through which smaller molecules and ions can move—carrying the heavy atoms with them. The spaces between and within them look ragged to one's eye. But clearly to approaching reactants they must offer specific attractions. So far as one can see, most of the heavy atom-containing molecules introduced are not chemically attached to particular residues but individually trapped in clefts of various shapes and sizes within and between the molecules.

While in each small region of the myoglobin crystal one can see operating the same stereochemical conditions that determine atomic arrangement in smaller crystals, it is much more difficult to recognize the principles that determine the pattern of chain fitting into the molecule as a whole. Yet principles there must be, complex as the pattern is. With small variations and at low resolution only, similar patterns have been observed both in seal myoglobin and horse methæmoglobin. The crystal structure of hæmoglobin is particularly interesting. Piecing together the low ridges of electron density observed in the three-dimensional electron density distribution, Perutz has found that each molecule is composed of four myoglobin-like molecules, packed together in an irregular tetrahedron. One might compare the packing here with Hooke's four globules were it not for the precise and intricate way in which chain locks into chain in the molecule as a whole. The iron atoms in the four hæm groups are found to be between 25 and 35 Å. apart. Already one can see detailed chemical implications of the structure. While their situation in clefts between chains provides a ready explanation for the slow reaction that has been observed of the iron atoms with bulky reactants, they seem to be rather far apart for any direct interaction. Possibly changes in the rate constants as oxygen is added arise from a general loosening of the molecule as reaction proceeds.

At this point it would be possible to discuss the building up of units of the size of hæmoglobin into still more complex molecules and crystals such as can be seen among the viruses. Poliomyelitis virus, for example, has been crystallized and gives very beautiful X-ray photographs. From these, Dr. Klug has deduced that the virus molecule is spherical and has an arrangement of sub-units over its surface which approximates to cubic symmetry. One of the formally possible arrangements seems very likely from the look of the electron microscope pictures. Others, still more complicated, have been found for other viruses.

In each of these enormous molecules there must be, at an atomic level, the same kind of intricate fitting together of individual atoms observed in simpler systems; and this leads us back to ask more intimate questions about the way in which the different molecules react with their surroundings. What, for example, is there in the chemistry of cobalt and iron that makes them specifically suitable for the biological reactions of vitamin B_{12} and hæm? In other fields of chemistry, new and most curious types of compound have been discovered, involving both cobalt and iron in hitherto unsuspected types of binding. The versatility of the chemical elements in their relations with one another is proving to be much greater than was supposed even a few years ago.

Looking back through history, one finds oneself impressed by the many characteristics of the molecular structures here described which were correctly inferred or imagined in the past, often from very indirect evidence. Yet these structures, as we have found them to be in detail, also contain new and surprising and undreamed-of features. The impact of the exact knowledge we now have is tremendous. It changes our whole outlook.

THE MANCHESTER LITERARY AND PHILOSOPHICAL SOCIETY

THE new buildings of the Manchester Literary and Philosophical Society in George Street, Manchester, were formally opened by Sir Cyril Hinshelwood, president of the Royal Society, on September 12 in the presence of the Lord Mayor of Manchester, who brought civic greetings and good wishes for the new venture. Prof. H. Lipson, president of the Society, who presided, referred to the past of the Society, which was indeed founded in 1781; but its premises with its irreplaceable library and records were destroyed by bombing in December 1940. He then commented that, although the Society now has its own building again, no society can live on its past. The membership of about 350 is little greater than in the time of Dalton and Joule, and Prof. Lipson hoped it would now increase and be maintained. The Society has a part to play in the life of Manchester that the University itself cannot hope to do.

Sir Cyril Hinshelwood, who had stoutly vindicated the function of the scientific society or academy in the conditions of to-day in his tercentenary address to the Royal Society, did not pursue that theme on this occasion; but, picking out the title of the Society and its original objective, "the advancement of literature and science", proceeded to discuss the arts and the sciences and, as he put it, to "roam destructively about, hacking at some of the useless conventional hedges which divide up liberal studies". It would appear, he began, that the division between the much-discussed 'Two Cultures' is not recognized in the Society, and the refusal to do so should be applauded and upheld: the dichotomy in question is largely illusory, but the illusion, in so far as it is believed or acted on, can do great mischief. So too can the false dichotomy between pure and applied science, and Sir Cyril suggested that it would be more sensible if people who took an intelligent interest in any of these things considered rather what they have in common than matters of difference. Inquiring further, Sir Cyril suggested that the philosopher, in the widest possible sense of the word, seeks some coherent vision of the world, something deemed worth while in its own right and worth the cultivation of skill and the exercise of effort to obtain. This, with the satisfaction of an urge to create, covers a large part of the liberal or philosophical or educated approach to existence, and the manifold ways in which it can find expression are only variations on the fundamental themes. The real antithesis to the liberal attitude lies in such things as the pursuit of power for its own sake, the accumulation of wealth except as a means to an end, or the basing of moral judgments on self-righteousness and prejudice.

Rejecting the view that the sciences deal with things and the arts with people, Sir Cyril insisted that both science and art are concerned with life, with living; the barriers between them are artificial, and in each alike there is a place for observation and experiment, for imagination and creative powers. Scientific imagination does not differ from any other kind and is called into play in science at least as often as elsewhere. Nor is the æsthetic element without an important role in science, though less commonly recognized, and to try to draw a distinction between the natural sciences and some of the arts in terms of objectivity can be equally misleading. Science is more than the simple enumeration of the facts of Nature: it is the ordering of these facts into patterns which are felt to be significant and satisfying, which can be interpreted and explained, and both involve subjective judgments. The acceptance or non-acceptance of a physical principle as a fundamental law of Nature must in the end rest largely on an æsthetic judgment.

Even in abstract art, Sir Cyril thought the analogy and affinity with pure mathematics are suggestive, nor did he think curiosity and the excitement of discovery operate differently or to different degrees in the arts and in the sciences. It is the weakest kind of sentimentality to make enjoyment of poetic images dependent on some desperate determination to accept them literally or to shun all other knowledge. Passing over the problems raised by history, whether it is a science or simply the register of the crimes, follies and misfortunes of mankind and its strivings and virtues, Sir Cyril commented that history does not teach us much about the handling of men. The sympathy and imagination needed to understand motives and feelings, the wisdom and experience to judge effect are generated not by the study of history but by the processes of real life. The real value of history, like that of science, is the ordering of complex arrays of facts into patterns to give a coherent though never wholly objective view of the world, and in both, as in art, the exercise of a strenuous and disciplined technique is essential.

Before considering technology, Sir Cyril made the comment in passing that there is no greater mistake, in science or in the fine arts, than to under-value manual craftsmanship, and in some respects the educational system of Britain seems designed to divert some with potentially first-rate manual skill into being second- or third-rate mental workers. At every stage of their development, fundamental science and technology have been interdependent. To-day no technological institute can dispense with the best theoretical equipment; but no laboratory devoted to pure science can hope to advance far without the finest technological assistance, and governments, industries and universities have still to find the best solution of the problems of organization which this situation presents. Moreover, many of the fundamental laws of physics confront us with metaphysical problems such as the nature of space and time, of causality and determinism, about which it is easy for one untrained in philosophical thought to make very unwise statements. Conversely, the philosopher who deals with these matters is in grave danger of spinning words in a vacuum, if not of perpetrating blunders, if he does so without know-

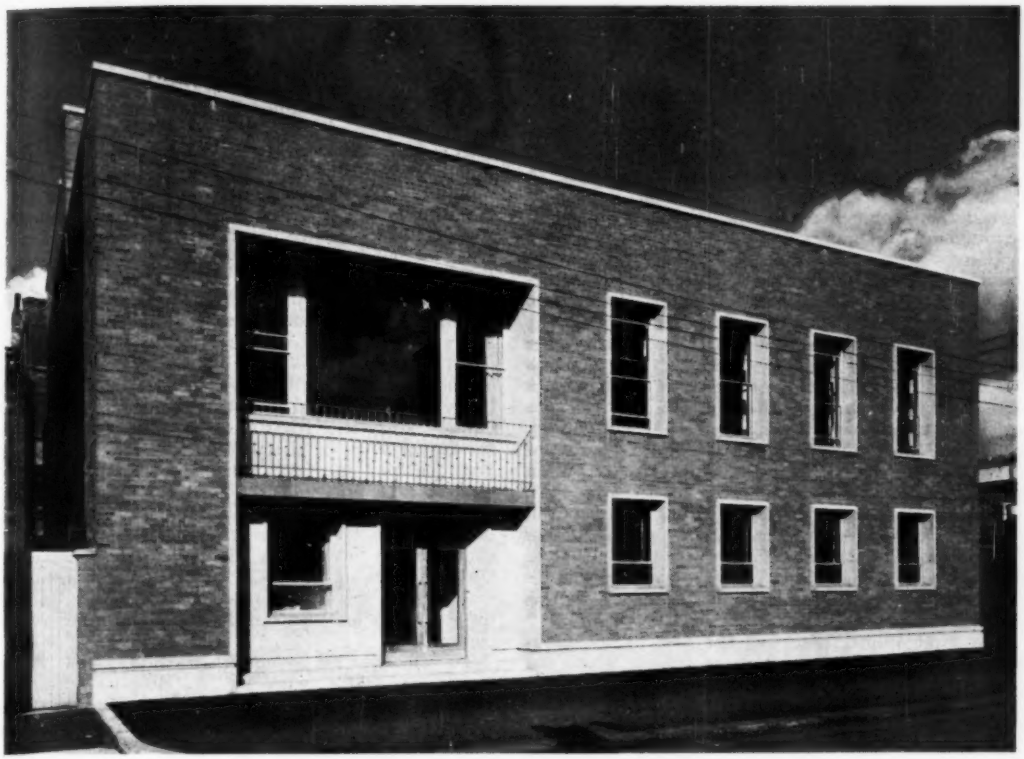

Fig. 1. New meeting house of the Manchester Literary and Philosophical Society

ledge of what physics has revealed. Biology, too, needs an occasional injection of philosophy and in the last resort physicists, philosophers, biologists, psychologists and even moralists ignore one another at their cost when confronted with some of the deepest problems of existence.

Finally, concluded Sir Cyril, the liberal arts and sciences have in common that they seek a coherent vision of the world, and that they all demand a highly disciplined technique. They are not inherently superior or inferior to one another, and the actual content of a particular study has little influence on the development of personality. Only real experience of the world exerts such influence, and it is the liberal approach rather than the choice itself that matters. Sir Cyril qualified this declaration of independence—to follow one's own bent and to respect others who follow theirs—by urging that arts and sciences should be cultivated side by side. They are allies, not rivals or antagonists; and he rejected the idea that the pressure of modern life and the demands of specialization make it impossible for the scientist to know something of literature and the arts man to know something of science. What matters in the end is the atmosphere, the attitude and the approach. The right ethos is likely to be found, he said, in the Manchester Literary and Philosophical Society, and he hoped it would be maintained and spread far and wide.

After a vote of thanks to Sir Cyril moved by Lord Simon of Wythenshawe, and conferment of honorary membership on Sir Cyril, the building was opened for inspection, and members of the Society may justly feel that the dignity and spaciousness which the architects, Messrs. Francis Jones and Sons of Manchester, have brought to their new home admirably fit it for the function which Sir Cyril commended. Besides the main lecture theatre on the ground floor, seating about 200, in which the opening meeting was held, with comfortable seating, good acoustics, and all the devices needed by a contemporary lecturer, it has on the first floor a smaller lecture theatre for more informal meetings of about 70, which can be arranged for social meetings also, and the Dalton Room, opening off which is the members' common room. The Dalton Room, which also serves as the council room, houses a small reference library and a collection of relics, mementoes and books relating to the history of the Society and its members, while the periodicals and other publications received from all over the world will be available in the common room, where light refreshments will be served. Although the Dalton Room holds pride of place, the whole building is light and appropriate to its contemporary purposes, and the equipment and furnishings are in keeping. Great credit is reflected on all responsible for the detailed planning and its execution.

OBITUARIES

Prof. F. J. M. Stratton, O.B.E., F.R.S.

A GREAT many friends all over the world will have learned with regret of the death on September 2 of F. J. M. Stratton; 'Professor' to some, 'Colonel' to others, 'Chubby' to many.

Frederick John Marrian Stratton was born in Birmingham on October 16, 1881. He was educated at King Edward's Grammar School, at Mason University College (afterwards University of Birmingham) and at Gonville and Caius College, Cambridge. He was third Wrangler in 1904, Isaac Newton Student in 1905, and Smith's Prizeman in 1906. In the years before 1914 he was a mathematics lecturer at Caius College and also assistant director of the Solar Physics Observatory, Cambridge, under H. F. Newall. His early papers covered a wide range of astronomy, including celestial mechanics, but the appearance of Nova Geminorum (1912) focused his attention on what proved to be a problem of life-long interest. In 1914 he had gone to the Crimea with Newall, intending to observe the total solar eclipse of August 21, but on the outbreak of war he hurried back to England and joined the Army immediately. He served with great distinction in France and was awarded the D.S.O. and the Légion d'Honneur.

The First World War was the first of the great upheavals of this century and the one for which the world was least prepared. At its end, Stratton returned to Caius College, as senior tutor, to face what was a difficult time of readjustment for the University at Cambridge, as much as for Britain as a whole. If one may judge by the remarks of his pupils it was a job for which his personality and experience admirably suited him, and which he carried out with much success. During this period he continued to give lectures on astronomy, among them one of the first general courses on astrophysics to be given in Britain. Somehow he also found time to go to Sumatra for the 1926 total solar eclipse, where with C. R. Davidson he made successful observations of the chromospheric spectrum.

On Newall's retirement in 1928, Stratton was appointed professor of astrophysics and director of the Solar Physics Observatory, relinquishing his tutorship at Caius. He held this post until 1947, although his tenure was interrupted, first by a serious illness in 1931 and later much more extensively by the Second World War. This period of his life was notable for the encouragement and help he gave to a whole generation of younger astronomers, many of whom still remember him with gratitude. He organized three more eclipse expeditions, to Siam, Canada and Japan, but was dogged by bad luck with the weather. Only in Japan, in 1936, were results of any scientific value possible. Even then the success was only partial, the Sun being covered by a cloud almost at the instant of second contact. However, members of his team made good measurements of wave-lengths in the spectrum from very near the Sun's limb, and obtained chromospheric spectrograms, study of which by Thackeray and by Woolley stimulated a good deal of later work.

In 1934, Nova Herculis appeared and, despite the inadequate equipment of the Solar Physics Observatory at Cambridge, Stratton and his staff during the next few months obtained a remarkable record of the spectrum changes of what turned out to be an extremely interesting star. Work on this spectrum absorbed much of his energies for several years, culminating in the production, with W. H. Manning, of the "Atlas of the Spectrum of Nova Herculis", using material made available from all over the world. This is still one of the most complete records we have of a nova outburst.

In 1939, at the outbreak of war, Stratton was bitterly disappointed at the refusal of the British Army to allow him to undertake active service again (at the age of fifty-seven), but nothing would stand in the way of his giving service of some kind. So the war years found him travelling extensively, in Canada, Australia, India and elsewhere, on duties for the Royal Corps of Signals. At the conclusion of the War, he had only two more years as professor of astrophysics, and in the conditions then prevailing in England, he realized he could do little to get back into active astronomy. He did, however, complete an interesting history of the Cambridge Observatories up to 1947. After his retirement he was deputy scientific adviser to the Army Council for two years and continued to serve on innumerable committees.

To celebrate his seventieth birthday, some of his pupils undertook, with Dr. A. Beer as editor, to produce what was to have been a *Festschrift*, but which expanded, as more and more of his friends came to hear of it, into two thick volumes, "Vistas in Astronomy". Indeed, the publication quite outgrew its original purpose, no doubt causing Stratton some amusement as well as gratification, and a third volume appeared, while yet others are still in preparation.

Stratton's official posts formed only a part of his activities. During 1925–35 he was general secretary of the International Astronomical Union, and did much to foster what was one of the earliest and most successful of the international scientific unions. He was also general secretary of the International Council of Scientific Unions from 1937 until 1952, and general secretary of the British Association, 1930–35. He was president of the Royal Astronomical Society, 1933–35, and its foreign secretary during 1945–55. He was honorary secretary of the Society for Visiting Scientists during 1948–55. He was president of Caius College, 1946–48, and at the time of his death was its senior Fellow. He served as president of the Society for Psychical Research, 1953–55. He was a life-long supporter of the Unitarian Church and was ever active on behalf of ex-servicemen's societies and causes. In the early 1930's he gave much help to refugee scientists from Central Europe. He was elected to the Royal Society in 1947.

Until his last years, Stratton lived life at the double. He thought fast, talked fast, decided fast, and in his younger days moved fast. Despite great sociableness and much hospitality, he was not an easy man to get to know really well, in part because he tended to keep his life in watertight compartments. He made great contributions to science and learning, chiefly by help and encouragement to younger men,

and not merely in Great Britain alone. A bachelor, he was completely devoted to his College, his pupils, to astronomy and especially to the International Astronomical Union, to his comrades of the First World War, and to his duty wherever he thought it to lie. Personal convenience, comfort or profit came very low indeed on his scale of priorities. He was one of the most generous men I have ever met, and one of the most modest. Astronomer, soldier, don, Unitarian, internationalist, he has left the world greatly in his debt.

R. O. Redman

Among young intellectuals in the universities, in the years before the First World War, it was commonly regarded as a sign of feebleness of intellect to believe that war was conceivably possible; and indeed as a mark of moral obliquity to take any personal steps to meet such an emergency. Fortunately a substantial minority, in reality no less intelligent, paid rather little attention to the intellectual and moral stigma; and the following short story of a small group of them at Cambridge describes one of the earliest contributions of young scientific men to national preparedness for war.

In 1908, under the Haldane Scheme for the Territorial Army, the Officers Training Corps of the Universities took the place of the earlier Volunteers. In Cambridge, in addition to the previous battalion of infantry, units were established of cavalry, artillery, engineers and medicals. Two years later, under the imaginative leadership of F. J. M. Stratton, a Signal Company was started and before long a wireless telegraph section was formed as part of it. Encouraged by Bertram Hopkinson, professor of engineering, who lent a room in his laboratory, the wireless section began work on the design of sets for field work and it drew in a remarkable group (as it proved) of young scientists for the task. It was not possible in those days to go and buy components from dealers, and radio valves did not exist. Everything had to be built up in workshop, laboratory, or tent, by members of the section itself. Since sets had to be portable, a generator was designed to be fitted to a bicycle, and during 'operations' a member of the section pedalled steadily, if wearily, to provide power for transmission. When communication failed, for example, between Farnborough Common and Eelmore Hill, it was re-established by flag and/or bicycle.

A photograph exists showing the ten members of the section in 1912, together with Captain Stratton commanding the Signal Company and a portable radio set complete with antenna. In it, among others, are Lance Corporal Henry Thirkill, Lance Corporal T. L. Eckersley, Private Harold Spencer Jones and Second Lieutenant A. V. Hill. Those were early days. By 1914 the wireless section had expanded considerably, and since no reserve of regular W/T officers existed when the War broke out, it was natural that members of the section should be drawn into field wireless. In fact, the section provided senior wireless officers for four armies. After the War, the Signal Company was more generously treated by the War Office, and a special research group was formed inside the wireless section.

Prof. (alias Lieut.-Colonel) Stratton recalled that, among the members of his Signal Company, the following could be counted: several who went into industry, one of whom was scientific adviser to Marconi's for twenty-seven years; four vice-chancellors and five masters of Cambridge colleges; nine professors; nine Fellows of the Royal Society and three Nobel Laureates; one director of scientific research, Admiralty; one chief engineer of posts and telegraphs, India; one secretary of the World Meteorological Organization; three successive secretaries-general of the International Council of Scientific Unions; one Astronomer Royal; two major-generals; [one D.S.O.]; one bishop; one Colonial chief justice; one M.P.; and one Olympic gold medallist. This list, as its author admitted, was rather a fraud, since on the average each person in it occurred twice. Nevertheless, it is rather impressive, particularly when one remembers that two-thirds of these people served in the Signal Company during its initial years 1910–14.

A. V. Hill

Dr. J. Brooks, O.B.E.

Dr. Jack Brooks, principal scientific officer at the Low Temperature Research Station, who died in Cambridge on October 2, was in charge of the work on eggs and poultry.

Brooks joined the scientific staff of the Low Temperature Research Station in 1928 from the Muspratt Laboratory of Physical and Electro-Chemistry of the University of Liverpool, at the invitation of the late Sir William Hardy. From the outset of his career he was an individual worker, belonging to the less-common class of investigator who prefers to do most things for himself in the laboratory. Before the War Brooks made a name for himself in fundamental research, especially in the field of hæmatin pigments. Quite early in his career he was publishing work in the *Proceedings of the Royal Society* and the *Biochemical Journal*, and up to the outbreak of war he continued to produce a steady series of papers in his particular field of physical chemistry applied to biological systems. He then showed himself equally capable at quite the highest level in applying theoretical results to immediate practical objectives, namely, the dehydration of egg and the storage of dried material.

Brooks held an established position as the leading Government consultant in Great Britain on all matters concerned with the handling and storing of eggs. During the period 1943–47 he was lent to the then Ministry of Food for various periods, in the course of which he visited the Argentine, United States, Canada and Australia in connexion with the Ministry's programme for the manufacture of dried egg and, afterwards, for full-scale trials of sugar-dried egg production. Later he again visited Australia as a member of the British Food Mission. After the War the emphasis of Brooks's work turned from dried to shell eggs, and again his knowledge, skill and personality were called on by the Ministry of Food to help in the solution of problems in countries exporting eggs to Britain. In 1948 and again in 1950, he visited Poland in response to a request from the Polish Government for help in finding a solution to difficulties which had arisen in the preparation of frozen pasteurized egg in fulfilment of orders placed by the Ministry of Food. Brooks was the only person in Britain possessing the specialized knowledge for this task, and he was able to discover the cause of the trouble and suggest a solution completely satisfactory both to the Polish Government and the Ministry.

Although such calls for Brooks's services seriously interrupted his supervision of laboratory investigations, he continued to exercise a firm control over

these and to initiate and maintain lines of research which have been successful in several directions. Some of these lines, such as the storage of oiled eggs, had immediate industrial application. Others were aimed at discovering the mechanism of deteriorative changes which occur during prolonged storage such as, for example, the thinning of the white, the weakening of the yolk membrane, and the production of undesirable changes in flavour due to enzymic oxidation. These were all problems needing deep exploration and a capacity for skilful research of the most fundamental nature. But for his deflexion from 1940 onwards to the more applied aspects of his work, Brooks would almost certainly have established for himself as high a reputation in academic circles as he enjoyed in responsible quarters for his technical skill and knowledge of matters pertaining to eggs.

E. C. Bate-Smith

NEWS and VIEWS

Air Ministry Appointments : Mr. Hayne Constant, C.B., C.B.E., F.R.S.

Mr. Hayne Constant has been appointed Scientific Adviser, Air Ministry. As a Fellow of the Royal Society and a member of the Aeronautical Research Council, and as director of the National Gas Turbine Establishment, Pyestock, he has for many years been closely connected with forward thinking in the aeronautical field. Born in 1904 at Gravesend, Kent, he went up to Queens' College, Cambridge, as a scholar in 1924 and graduated with first-class honours in the Mechanical Science Tripos in 1927. After a year's postgraduate research he entered the Royal Aircraft Establishment, and, after work on aircraft vibration and the performance of engines on weak mixtures, he pioneered work on the axial jet engine. His career has been mainly devoted to the advancement of the gas turbine as a prime mover, particularly for aircraft, in which sphere there has been such enormous advance in the past twenty years. In 1941, Hayne Constant was head of the Engine Department of the Royal Aircraft Establishment. In 1944, he linked forces with the Power Jets Whittle team as Power Jets, Research and Development, Ltd., and when that body became the National Gas Turbine Establishment he was deputy director. In 1948, he was elected a Fellow of the Royal Society and in the same year he succeeded Dr. (now Sir Harold) Roxbee Cox as director. In this capacity he was responsible not only for research but also for the centralization of the National Gas Turbine Establishment, bringing the Whetstone (Leicester) Whittle group to Pyestock, and the development of Pyestock into the biggest centre in Britain for gas turbine and ramjet test plant, including the Engine Test Facility. He received the Busk Memorial Prize of the Royal Aeronautical Society in 1932, and the Clayton Memorial Prize of the Institution of Mechanical Engineers in 1947. He was, from its inception in the war years, a member of the Gas Turbine Collaboration Committee with senior representatives of the British aero-engine industry. His views of, and predictions for, the gas turbine are published in his book "Gas Turbines and Their Problems", and in lectures and papers in many scientific and technical journals.

Mr. R. H. Weir, C.B.

Mr. Robert Hendry Weir has been appointed director of the National Gas Turbine Establishment in succession to Mr. H. Constant. Mr. Weir, who has been principal director and director general of engine research and development since January 1, 1954, was born in 1912 and educated at Allan Glens School, Glasgow, and the University of Glasgow, where he graduated B.Sc. with first-class honours in mechanical engineering in 1933. After an apprenticeship with Wm. Denny and Bros. Ltd., Dumbarton, he joined the Royal Aircraft Establishment in November 1933, where he was a member of the Engine Department until January 1939. He was then transferred to a post at headquarters in London, where he was engaged on aero-engine performance and work on pressurized cabins until December 1940. His next posting was to the Aircraft and Armament Experimental Establishment, Boscombe Down, where he spent a period of eighteen months on aero-engine liaison duties, and then transferred back to Engine Research and Development Headquarters in July 1942, for special duties on gas turbines. Mr. Weir was appointed director of industrial gas turbines in February 1950, and director of engine research and development in August 1952. He was made principal director of engine research and development in January 1954, which post was re-named director general of engine research and development in January 1959.

Ministry of Health : Sir John Charles, K.C.B.

Sir John Charles, chief medical officer of the Ministry of Health, Ministry of Education and Home Office, is retiring on November 30. He is a graduate of the University of Durham, and achieved a high reputation as medical officer of health, Newcastle on Tyne. In addition to competent administration, he made important epidemiological, nutritional and public health inquiries. During the Second World War he was appointed a deputy chief medical officer of the Ministry of Health and in 1950 became chief medical officer. In Sir John's ten years of office he has given much attention to the development and consolidation of the National Health Service. He has seen a further notable decline in tuberculosis and diphtheria, and has encouraged effective vaccination against poliomyelitis. Among many other subjects which have engaged his attention are the mental health services under the new Act, the dental services, care of the aged and the progress of maternity and child welfare work. Sir John has visited various parts of the world in the interests of international health, being also chairman of the Executive Board of World Health Organization and president of the twelfth World Health Assembly. He gave the Bradshaw Lecture in 1948 and the Harveian Oration in 1955 to the Royal College of Physicians. He can look back with satisfaction on his tenure of office as medical adviser to three departments of State ; and in his retirement

may find time for further studies in medical history, which is one of his interests.

Dr. G. E. Godber, C.B.

Dr. George Edward Godber has been recently appointed to succeed Sir John Charles, with effect from December 1. Dr. Godber was educated at Bedford School and New College, Oxford, where he read physiology, and at the London Hospital. After qualifying, he served for twelve months as house physician in the professorial unit under Sir Arthur Ellis. Subsequent to gaining some experience of general practice and of local government service in Surrey, he joined the staff of the Ministry of Health in 1939 at the age of thirty. His first assignments there were concerned with the reception of evacuees in the reception areas and with the other early wartime responsibilities which fell to be discharged by the Ministry. Later he was responsible with the late Sir Leonard Parsons and Mr. Clayton Fryers for carrying out the combined Ministry of Health and Nuffield Foundation survey of hospitals in the Sheffield Region. Returning to Whitehall, he was concerned in association with other members of the Ministry's lay and medical staff in the various preparations which culminated in the launching of the National Health Service on July 5, 1948. Since he was appointed as deputy chief medical officer, his chief responsibilities have continued to lie with the several branches of the health service, and he has been active in collaborating both with the College of General Practitioners and the Society of Medical Officers of Health. He has been and continues a vigorous advocate of home care of the sick wherever practicable, and of active co-operation between general practitioners, local health authorities and the hospital and specialist services.

The Royal Society of Edinburgh: Officers for 1960-61

At a statutory meeting of the Royal Society of Edinburgh held on October 24, the following officers and members of Council were elected: *President*, Prof. E. L. Hirst; *Vice-Presidents*, Prof. T. Neville George, Prof. J. R. Matthews, Dr. T. R. Bolam, Dr. Douglas Guthrie, Dr. A. W. Greenwood, Prof. R. A. Rankin; *General Secretary*, Prof. Norman Feather; *Secretaries to Ordinary Meetings*, Dr. Mowbray Ritchie, Prof. A. E. Ritchie; *Treasurer*, Dr. J. R. Peddie; *Curator of Library and Museum*, Dr. R. Schlapp; *Members of Council*, Prof. E. G. Cullwick, Prof. G. Pontecorvo, Prof. M. M. Swann, Prof. V. C. Wynne-Edwards, Prof. H. A. Brück, Dr. H. R. Fletcher, Prof. G. L. Montgomery, Prof. W. L. Weipers, Prof. J. H. Burnett, Prof. C. F. Davidson, Prof. D. C. Pack, Dr. A. B. Stewart.

Applications of Electricity in Aircraft

The Councils of the Institution of Electrical Engineers and of the Royal Aeronautical Society have recognized the growing need to provide a forum wherein engineers engaged respectively in the fields of electrical engineering and of aircraft construction can meet to discuss problems and new ideas affecting their common interest in aircraft electrical engineering. A Joint Committee, of which Prof. G. A. Whitfield, of the College of Aeronautics, Cranfield, is the chairman, was recently formed by the two organizations to consider ways and means of stimulating discussion of these matters, and it has now been agreed that this can best be done by arranging for joint meetings of the two organizations in London and for the formation of joint groups in the provinces. It is intended that meetings of the joint groups should be informal and in the nature of colloquia, designed to interest technicians as well as aeronautical and electrical engineers. In order to launch the scheme there will be an inaugural meeting at the Institution of Electrical Engineers, Savoy Place, London, W.C.2, on November 11, at 5.30 p.m., when Viscount Caldecote will deliver a lecture on "The Future of 'Electrics' and 'Electronics' in Aircraft and Guided Missiles".

Department of Scientific and Industrial Research: Report on Shipbuilding

In answer to questions in the House of Commons on October 27, regarding the publication of the Council for Scientific and Industrial Research's report on shipbuilding, Sir David Eccles, as representing the Minister for Science, said that the report was the subject of confidential discussions between the Council and representatives of the Ministry of Transport and the industry. The question of publication, which was one for the Council, would not arise so long as these discussions were in progress, but the Council intended to publish a statement as soon as these discussions were concluded. In reply to a further question, Sir David said that the current grant to the Parsons and Marine Engineering Turbine Research and Development Association amounted to £70,000 in consideration of the £210,000 raised by the industry, and the grant for next year would be on the same terms.

Russian Chemical Reviews

The Russian *Uspekhi Khimii* is being published in translation as *Russian Chemical Reviews* by the Chemical Society with the support of the Department of Scientific and Industrial Research. The first issue has appeared and contains four review articles, on the chemical action of ultrasonic waves on macromolecules, developments in the chlorination of saturated hydrocarbons, the hydrides of the rare-earth metals, and the chemistry of quinuclidine derivatives. These are excellent surveys of the subjects, with extensive references to papers in Russian and other languages, and the translation appears to have been very well done. The translation is described as cover-to-cover, but reviews in the original which are translations from foreign periodicals are, with few exceptions (for example, Chinese sources), not included. The subscription rates are £12 (United States 36 dollars), with a reduced rate for libraries of universities and technical colleges of £9 (27 dollars) per annum, including postage. Orders should be sent to Cleaver-Hume Press, Ltd., 31 Wright's Lane, London, W.8.

Checking Accidents in Factories

Under the title "Guide to Statistics collected by H.M. Factory Inspectorate" the Ministry of Labour has issued a booklet giving details of the methods of collection and compilation of the statistics, and of the systems of coding and classification which permit analysis of further information as and when required (Pp. i+41. (London: H.M. Stationery Office, 1960.) 4*s*. net). These statistics are based on the reports of accidents made by occupiers of factories and by certain other employers in accordance with

Section 64 of the Factories Act, 1937. The circumstances in which reports are required are detailed, and copies of the prescribed forms are appended to the booklet and also of the tables used for classifying the information. The booklet describes in detail how this information is examined, classified and given a coding to permit subsequent analysis.

Grants for Field Work in Natural History

Groups of local naturalists often find themselves held up for lack of specialist advice on how to tackle their problems. To help its members, the Council for Nature approached the Carnegie U.K. Trust, which has now agreed to set aside a fund from which it will make grants to approved societies for assistance with field work. These grants will be made on recommendations submitted by the Council for Nature and will be contributions towards expenditure incurred in carrying out specific projects that give amateurs experience of field work in natural history under skilled direction. Approved expenditure is likely to include fees paid to experts for instruction and leadership, the incidental expenses of such people and the cost of essential equipment subject to certain conditions. Grants will not be available for the expenses incurred by members of societies taking part in any scheme or for the general support of a society. Full particulars of the conditions for the award of grants can be obtained from the Carnegie U.K. Trust, Comely Park House, Dunfermline, Fife, or from the Council for Nature, 41 Queen's Gate, London, S.W.7.

The Wellcome Trust : Grants for Medical Research

During the six months from March 1 to August 31 the Wellcome Trustees accepted the following applications for major grants to assist medical research. Together with smaller grants totalling £41,000, and Wellcome research travel grants, totalling £17,000, the allocations by the Trustees in this period exceeded £420,000. The grants were allocated as follows: £50,000 to the Middlesex Hospital Medical School, University of London (Prof. C. A. Keele), for a suite of Wellcome Research Laboratories in the Pharmacology Department of the new Medical School building; £50,000 to the Massachusetts General Hospital, Boston, to establish a Wellcome research professorship for ten years; £61,000 to the Medical Research Council, to construct a laboratory and housing accommodation for an Epidemiological Research Unit in Jamaica, also £60,000 to provide five Sir Henry Wellcome research travelling fellowships for five years; £40,000 to McGill University, Montreal, Canada, to extend the support of the Wellcome Research Department of Anæsthesia for a further five years; £17,000 to the University of Pennsylvania, to extend the support of the Wellcome associate professorship of research in anæsthesiology for a further three years; £16,000 to Trinity College, Dublin (Mr. K. M. Shaw), to construct and equip an experimental surgery unit and an animal house; £16,300 to the University of Oxford (Sir George Pickering), to construct an animal house for the use of workers in the Department of the regius professor of medicine; £6,000 to the Postgraduate Medical School, University of London (Prof. J. McMichael), to equip a cardiac catheterization research laboratory in the Department of Medicine; £10,000 to the University of Birmingham, Department of Biochemistry (Prof. S. V. Perry), for equipment to investigate the nature and functions of the proteins of brain and muscle; £5,500 to St. Mary's Hospital Medical School, University of London (Prof. R. R. Porter), for an analytical ultracentrifuge to investigate the structure of antigens and antibodies.

In addition the Trustees made grants totalling £21,800 for special research apparatus of various kinds to Prof. R. Woolmer, Department of Anæsthesia, Royal College of Surgeons of England; Dr. G. A. Kerkut, University of Southampton; Mr. H. A. Sissons, Institute of Orthopædics, University of London; the Agricultural Research Council's Virus Research Unit, Cambridge; Dr. K. S. Dodgson, Department of Biochemistry, University College of South Wales and Monmouthshire; Prof. O. L. Wade, The Queen's University, Belfast; Prof. G. A. H. Buttle, School of Pharmacy, University of London; Prof. J. W. Howie, University of Glasgow; and Prof. H. Heller, University of Bristol. A grant of £1,000 to purchase special equipment for high-altitude research was made to Dr. L. G. C. E. Pugh, physiologist to the Himalayan Scientific and Mountaineering Expedition, 1960–61.

Announcements

Prof. Arne Tiselius, of the University of Uppsala, Nobel laureate in chemistry (1948), has been appointed president of the Nobel Foundation.

The Medical Research Council has recently established the following new research units: the Gastroenterology Research Unit at the Central Middlesex Hospital under the direction of Dr. E. N. Rowlands; and the Unit for Research on the Chemical Pathology of Mental Disorders in the Department of Physiology, University of Birmingham, under the honorary direction of Prof. I. E. Bush.

The Institute of Biology, with the support of the Eugenics Society, has instituted an annual Darwin Memorial Lecture in human biology. The first lecture will be given by Dr. C. E. Ford of the Medical Research Council's Radiobiological Research Unit on "The Cytogenetics of Sex in Man". The lecture will be given in the lecture hall of the British Museum (Natural History), Cromwell Road, S.W.7, at 5.15 p.m. on November 23 and is open to the public.

The annual exhibition of the Institute of Physics and the Physical Society will be held at the Royal Horticultural Society's Old and New Halls, Westminster, London, S.W.1, during January 16–20, 1961. During the exhibition, the following discourses and demonstration lectures will be given in the New Hall: January 17, "Hydrodynamics Research", by F. S. Burt (Admiralty Research Laboratory); January 18, "The Physics of the Ocean", by Dr. G. E. R. Deacon (National Institute of Oceanography); January 19, "Some Physical Problems in Travelling at Supersonic Speed", by Dr. F. P. Bowden (Laboratory for the Physics and Chemistry of Solids, Cambridge). Further information and tickets can be obtained from the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

Erratum. We regret that in the communication entitled "Evidence for the Identity of Natriferin, the Frog Water-Balance Principle and Arginine Vasotocin", by Prof. Wilbur H. Sawyer, in *Nature* of September 17, p. 1030, there is a mistake in the table; the right-hand heading should read: "Activities (u./mgm. dry weight)".

SCIENCE BY THE UPPER FORM IN SCHOOLS

At the annual meeting of the British Association for the Advancement of Science the section devoted to matters of general scientific interest, Section X (Assembly of Corresponding Societies), has for some years provided an opportunity for sixth-form scientists in local grammar and public schools to present papers on scientific studies carried out as a result of group or individual investigation. One whole morning session is set aside to allow the presentation of four topics, and large adult audiences flock to listen to the young embryo scientists.

This year at Cardiff, "Science by the Upper Form" was represented by Robert Riden, of Cardiff High School for Boys, who read a paper on "The Caves of South Wales"; Colin Rees, of Cathays High School for Boys, who discussed "The Increase of Herring Gulls at Worms Head, Gower, and its Effects on the Razor Bill and Guillemot Populations"; Glanffrwd Phillips and Clive Davies, of Ferndale Grammar School, Glamorgan, who presented a joint report on investigations into the presence of "*Craspedacusta sowerbii*—Fresh Water Medusa of Microhydra" in Darran Lake, Ferndale, and by Roger Williams, of Tredegar Grammar School, Monmouth, who surveyed the vast subject of "Astronomy" in half an hour.

Each talk was admirably illustrated with slides or projected photographs as well as by other visual material.

Robert Riden gave a vivid account of his adventures in exploring a number of caves of different types which border the South Wales Coalfield. He indicated their relationship to the geology of the coalfield, gave details of their structure and physical grandeur, with particular reference to three to be found in Breconshire, in Carmarthenshire and in Gower, and showed how young people with a spirit of adventure and a disregard for the discomforts to be faced could carry out much useful exploration underground, providing their expeditions were properly organized.

Colin Rees, with the help of some delightful coloured slides, exposed the ruthless habits of the herring gull on Worms Head in the Gower Peninsula. He unfolded an interesting story of variations in the population of bird life on the headland over the past twenty years, beginning with the rapid decline of the gull population during the war years due to human raids on the eggs as an additional item of food until rationing came to an end in 1947. The eggs of razor bills and guillemots escaped as they nested on the inaccessible cliff face, so that these birds multiplied at the expense of the herring gulls. But when the eggs of the herring gull were no longer in demand it reasserted itself very rapidly, laying, as it does, three times as many eggs a year and nesting wherever it can at the expense of the razor bills and guillemots, until herring gulls were once more swarming over the headland by 1959. To-day, Colin Rees stated, there are 150 pairs of herring gull to about 100 pairs of guillemots and 50 pairs of razor bills—he considered that the herring gull would go on increasing while the others would remain in equilibrium. He gave some interesting information about the feeding and nesting habits of the various birds, discussed the dangers of oil pollution, and showed how the prolonged and intensive study of one particular area of a coastline can bring much valuable information about the habits in general of certain birds, the inter-relationships between the species, and the effects of human interference on their lives and numbers.

The third topic of the morning concerning the freshwater medusæ found in 1959 in the Darran Lake, Ferndale, was illustrated by some excellent diagrams prepared by Pamela Evans and Moira Morgan of the same school as the speakers, Glanffrwd Phillips and Clive Davies. There was evidence throughout of careful preparation and good group organization by the senior biology mistress, Miss M. G. Lewis, who had also been responsible for the investigations in the field. The paper gave an account of the discovery of small, transparent jelly-fish-like creatures in the Lake near the school at the height of the very dry summer of 1959. At first thought to be sea urchins, the creatures were eventually identified as medusæ of *Microhydra*. Evidence was found of their previous location in other parts of Britain on rather rare occasions, always associated with hot, dry summers, but no clues could be discovered to account for their appearance at Ferndale in spite of much careful investigation of all the relevant factors. The series of slides revealed the wonders of the structure and variations in the shape of the medusæ and showed how they reproduced by forming simple buds. Microscopic specimens were also available for examination. It was a well-presented topic, indicating how much useful research can be carried out as the result of the chance discovery of one small strange creature in a lake.

The final paper was a discourse on "Astronomy" by Roger Williams. It was a quite masterly survey of this vast subject within the short time at his disposal and ranged from the history of the science to the analysis of the rival theories of the origin of the universe—"the explosive hypothesis" and the theory of continuous creation. Without being drawn into any religious controversy, Roger Williams pointed out that it is "no more absurd to postulate the supernatural as always creating than to contain the creative act to a single instant". He went on to consider the techniques at the disposal of the modern astronomer, referred to the importance of the work of the amateur, and thought that while inter-planetary exploration by man could not be far away it was not likely to provide us with many new clues as to the origins and structure of the universe. The pursuit of research into its mysteries will long remain of the utmost import to the pure astronomer, but Roger Williams concluded that the problem of what to believe and what not to believe must always remain a matter for the individual, unmodified by scientific knowledge.

Thus the session, "Science by the Upper Form", ended on a philosophical note. It is good to know that some sixth formers not only study their text-books, and listen to the experts, but also carry out their own investigations and learn to think for themselves. In encouraging these habits among our young scientists still at school, the British Association in this and many other ways is doing a notable work for the better understanding of science by the younger generation. "Science by the Upper Form" to-day may be the basis for scientific advancement to-morrow.

G. S. CAMPBELL

THE TENTH CANADIAN HIGH POLYMER FORUM

ON September 7, some 170 scientists drawn mainly from Canada and the United States began a two and a half day conference on natural and synthetic macromolecules, under the general chairmanship of Dr. L. A. McLeod. As Dr. Sivertz reminded the conference, the study of high polymers in Canada, which was already important in relation to the large pulp and paper industry, received further impetus when, in 1942, Canada became a joint partner with the United States in the manufacture of synthetic rubber. A small working party drawn from the universities and industry was then set up, and from this developed open conferences and finally the Canadian High Polymer Forum was constituted and held its first meeting in 1949. Since that date meetings have been held at almost yearly intervals, and these have been characterized by informal and intimate discussion of contributed papers on a variety of topics. The meeting reported on here was the tenth in the series, and among other activities was marked by a banquet at which the mood of the principal speakers combined reminiscence with planning for the future.

Perhaps an outsider may be permitted to say that the meetings have not only served their regional and national purpose with particular success but have also been of wider value and importance, and that it is therefore entirely appropriate that the Canadian High Polymer Forum should be host to the International Symposium on Macromolecular Chemistry at Montreal on July 27–August 1, 1961. The relative merits of the small discussion group and the large conference with unrestricted membership have often been argued[1] and it will not be surprising if on the basis of its experience of earlier forums the organizing committee attempts to preserve the advantages of the former at the Montreal meeting. The eleventh Forum will not be held until the autumn of 1962 or the spring of 1963.

Twenty-four papers, all of which cannot be mentioned here, were presented at the tenth Forum. Five of these were concerned with ionic polymerization of unsaturated hydrocarbons. From these it appeared that rate constants for some of the individual steps in both cationic and anionic polymerization are now becoming available and it should not be long before coherent kinetic and mechanistic patterns emerge. Thus Dr. P. H. Plesch gave a value for k_p for the titanium chloride catalysed polymerization of *iso*butene in methylene chloride at − 60° C. and values of the Arrhenius parameters of the spontaneous termination, monomer transfer and water transfer reactions relative to those for the propagation reaction, from which it appeared that monomer transfer is the last chain-breaking reaction to survive at low temperatures. Dr. A. M. Eastham gave an account of interesting attempts to study the initiation reaction free from the complications of propagation by an investigation of the kinetics of the geometrical isomerization of but-2-ene in ethylene dichloride induced by boron trifluoride–methanol mixtures. The results seem to show rather surprisingly that an adduct of but-2-ene, methanol and two molecules of boron trifluoride may be involved. In a rare, but most welcome, contribution from one of the pioneers (R. M. Thomas) of cationic polymerization three new important findings were reported. The first was a medium for achieving liquid-phase cationic polymerization of *iso*butene at − 185°, the second the demonstration by carbon-14 tracer techniques of solvent-fragment incorporation in the polymer and the third was the existence of an 'inversion' temperature of about − 45°, above which the degree of polymerization of poly*iso*butene produced by aluminium chloride catalysis increases with monomer concentration and below which it decreases. This last result provoked much discussion, and mechanisms of chain termination involving two monomer molecules were advanced. Prof. M. Szwarc described a capillary flow method involving contact times in the range 0·2–1·5 sec. for measuring the k_p for anionic polymerization of styrene in tetrahydrofuran solution with Na^+ as the counter ion, and also the preliminary results obtained, together with the relative efficiency of water and methyl iodide as chain terminators. An elegant spectrophotometric method for measuring the relatively slow reaction of lithium butyl with styrene was described by Drs. S. Bywater and D. J. Worsfold. The definite but unexpected result was obtained that, contrary to earlier work, the rate of the initiation reaction is proportional to $[\text{styrene}]^{1\cdot0}$ $[\text{Li Bu}]^{0\cdot15}$, whereas the rate of propagation is proportional to $[\text{styrene}]^{1\cdot0}$ $[\text{Bu (styrene)}_{\bar{n}}\ \text{Li}^+]^{0\cdot5}$. It seems that a six-fold aggregate of lithium butyl and bimolecular association of growing Bu $(\text{styrene})_{\bar{n}}$ Li^+ ion pairs must be involved in reactions in the lithium butyl catalysed polymerization of styrene. The latter association was much discussed in relation to earlier and later contributions (especially that of N. Nikolov) and generally agreed to be important in more concentrated solutions.

Free-radical processes received due measure of attention particularly in relation to unusual phenomena in solid systems. Thus the effect of sulphur-containing antioxidants is promoted not only by carbon black but also by polyacenes, diphenylpolyenes and substances capable of generating quinonoid structures. The effect was ascribed by the Bell Telephone Laboratory workers to formation of π-bond complexes between the promoter as acceptor and thiyl radicals as donors, thereby reducing the reactivity of the latter as hydrogen abstracting agents. Dr. R. J. Ceresa showed that the kinetics of the swelling of chemically cross-linked or molecularly entangled polymers by vapours followed similar courses, showing discontinuities to be associated with mechanical rupture of chains or the cross-links since the use of vinyl monomers as swelling agents leads to the formation of block copolymers. The hitherto somewhat puzzling phenomena connected with the polymerization of vinyl monomers in aqueous solution were discussed, and shown to be approaching rational codification based on the mutual compatibilities and the water solubilities of monomer and polymer, and the susceptibility of soluble polyradicals to oxidative and reductive termination by ions of metals of variable valency. The rate constants of the growth and termination processes of the soluble

radicals are not too dissimilar from those in non-aqueous media and are quite independent of those of any longer-lived radicals in suspended polymer particles.

Full characterization of a solution of a polymer requires a knowledge of the structure, sizes and shapes of all the polymer molecules present and of its equilibrium properties as manifested in partial molal values of thermodynamic functions of solvent and solute. Many of the papers were concerned with this topic in one or other form. One, by Drs. W. H. Beattie and J. T. Bailey, was a timely cautionary reminder, generally acknowledged, of the need for a sharpening of our perception of the significance of the number, weight, Z and $Z + 1$ averages obtained from light scattering. Another, by Dr. W. Cooper and his associates of the Dunlop Rubber Co., Ltd., showed that second-order virial coefficients obtained from light scattering measurements on polybutadiene are strongly dependent on chain branching and revealed that the branch points are preferentially susceptible to mechanical breakdown. One of the most exciting contributions was that in which Prof. W. Heller of Wayne State University described his attempts to "walk around" macromolecular particles oriented in a streaming solution by observing the light which they scatter when illuminated from chosen directions. Already this fascinating but technically very difficult approach has not only indicated the shape of rigid particles but has also given insight into molecular elasticity by providing indications of the change of shape of flexible coiled macromolecules under the influence of shearing forces. If this method could possibly be extended to the use of polarized light an immensely powerful tool would become available. A further paper on polymer solutions by Dr. B. E. Conway dealt with the thermodynamics of the polar system polyoxypropylene glycol in methanol. The decrease in hydroxyl content with increase in molecular weight of the polyoxypropylene glycol leads to a change of sign in the heat of mixing; the experimental entropies and free energies of mixing can be accounted for by the Münster theory, which takes into account specific solvent orientation effects.

A number of the authors described the application of new experimental techniques to the solution of polymer problems. Dr. L. A. McLeod has fractionated elastomers such as *cis*-1,4-polybutadiene using elution chromatography, and Dr. Bacon Ke showed that differential thermal analysis is not only useful in determining transition temperatures and heats of fusion of simple polyamides but may also give information concerning the structures of copolymers. Dr. W. Prins described a versatile osmometer which is capable, among other things, of measuring the de-swelling of polyelectrolyte gels. In this application the gel and solvent are separated by a porous stainless steel disk of porosity 5–65μ, and the change in gel volume is measured when pressures of up to 200 atmospheres are applied to the gel phase.

Some interesting developments in the field of new polymers and copolymers were described. The strong and highly extensible elastomers produced by the incorporation of 2,4-toluene diamine in copolymers of polyoxypropylene glycol and 2,4-toluene di*iso*-cyanate were discussed by A. J. Havlik of the California Institute of Technology. Poly (5-methyl, 3-vinyl, oxazolidine-2-one), of which the surprising properties in water were reported by W. E. Walles at the ninth Forum, forms strong complexes with a wide variety of organic compounds. The complex with phenol in which one phenol molecule may be bound per monomer unit in the polymer owes its strength to hydrogen bonding, but more unusual are the fairly strong complexes of the polyoxazolidinone with methyl bromide and bromine.

It is a tradition of these meetings to have a guest speaker who delivers the Conference lecture. This year the speaker was Prof. F. S. Dainton and his topic was "Why Polymerization Occurs". The central theme was that addition polymerization, which is usually regarded only from the point of view of the kineticist, is merely the aggregation of freely translating monomer molecules into larger molecules in which the basic units are joined by primary chemical valencies. Except in the nature and strength of the inter-unit linkage it is thus entirely analogous to physical aggregation processes such as condensation and crystallization and like these can be examined thermodynamically. The now familiar[2] notions of 'ceiling' and 'floor' temperatures, monomer–polymer equilibria and metastability phenomena were treated by the lecturer in relation to a wide range of systems. Recent calorimetric values of heats of polymerization and the entropies of polymers were used to illustrate the large effects on the heats and the minor effects on the entropies of polymerization of certain structural groupings in the monomer.

F. S. Dainton
K. E. Russell

[1] *Nature*, **184**, 835 (1959); **186**, 283 (1960).
[2] See Dainton, F. S., and Ivin, K. J., *Quart. Rev.*, **12**, 61 (1958).

PHILOSOPHY OF SCIENCE

THE British Society for the Philosophy of Science held its fifth annual conference at Wills Hall, University of Bristol, during September 23–25. The four formal meetings and discussions, organized by Dr. Mary Hesse (Cambridge), were attended by fifty members and guests.

At the first meeting, with Prof. H. Heilbronn (Bristol) in the chair, two papers on the relation between pure and applied mathematics were read. Accepting the views of Frege, Cantor and others that the concepts of pure mathematics are exact, and of Waismann and others that empirical concepts are inexact, Prof. S. Körner (Bristol) introduced a new generalized logic which embraced relations not only between exact concepts but also between inexact concepts and between exact and inexact concepts. He showed that 'purely exact' and 'internally inexact' concepts, as he defined them, could be identified with pure mathematical and empirical concepts respectively, and that they were logically unconnected. He thus described the application of mathematics as a threefold process: inexact empirical concepts are

replaced by exact concepts, exact conclusions are then deduced and, finally, the exact mathematical concepts are replaced by empirical concepts.

Prof. R. L. Goodstein (Leicester) traced the evolution of formal systems, using the development of geometry, mechanics and arithmetic to exemplify his thesis. The stages of evolution were successively the formulation and application of rules, the discovery of relations between rules and development of the concept of proof, the analysis of the rules of proof (metamathematics) and, finally, formal codification of metamathematics. He concluded that in applying mathematics we retrace its evolutionary development, replacing theorems by statements of observation. A mathematical proposition thus has a dual role. The ensuing lively discussion centred on the part to be taken by Körner's generalized logic in clarifying the problem, and on the strangeness of the fact that the exact concepts of pure mathematics could sometimes be successfully applied to the 'open-textured' concepts of empirical science.

Echoes of this first discussion were heard next morning when the theme was the relation between universality and necessity. Mr. W. C. Kneale (Exeter College, Oxford) gave a critical historical review of the question whether necessity can be defined by reference to universality. He traced three attempts at such definition: that, derived from Hume, in which necessity appears as a by-product of arguments the major premises of which are universal statements about Nature; that, derived from Bolzano and recently revived by Tarski, in which necessity is based on the universal validity of patterns of argument in which the only essential words are logical; and that, propounded by Popper in an appendix to "The Logic of Scientific Discovery", in which a Leibnizian reference to possible worlds seems to be related to a modified Bolzano–Tarski theory. Having explained the difficulties he found in all these reductionist programmes, Kneale advanced his own views that formal logic is pure theory of necessitation, that necessity is a fundamental notion, and that (with Wittgenstein) "all necessity is logical".

The second paper was given by Dr. I. Lakatos (London School of Economics), who first analysed the differences between the arguments of Popper and Kneale on both the ontological and epistemological planes. His witty theological formulation of the controversy, in which he sided more with Popper than with Kneale, included the startling credo that "the natural laws uttered by God were of infinite length" (and that therefore all universal statements, whether contingent or necessary, are false) and then he summarily dismissed the controversy as metaphysical and therefore meaningless. As a fallibilist, not only in science but also in logic and mathematics also, he could only regard both Popper and Kneale as mistaken in their "epistemological optimism", different though the forms of their optimism might be.

The chairman, Mr. J. W. N. Watkins (London School of Economics), then offered for discussion a model neatly designed to emphasize the conflicting views that had been propounded. In discussion, attempts were made to clarify the relation between logical and physical necessity. Do they form a linear continuum? Or are they homogeneous? But before complete clarification had been achieved, Dr. Lakatos silenced all further discussion by proving that any proof refutes the proposition it proves!

After strenuous discussion of closely knit philosophical arguments, the members turned with relief and pleasure to view the intriguing optical devices used by Mrs. M. L. J. Abercrombie (Guy's Hospital) to illustrate her paper on learning to perceive. The chairman was Prof. J. E. Harris (Bristol). Mrs. Abercrombie explained that perception is a process of interaction between the observer's sensory input and his store of previously acquired information. In this interaction only parts of the total information available are used by the observer, selection being made both from the field observed (clues) and from the store (schemata). The observer makes a guess about what he perceives, acts on his guess and modifies it as other clues and other relevant schemata interact. She described how training to improve perception is possible by reference to her recent experiments in training medical students to improve their skill in diagnosis.

The second paper was given by Dr. M. H. Pirenne (Oxford), who described his experiments to determine the threshold intensity of light visible to the human eye. The physical and psychological factors impeding exact measurements were explained. The subject's eye had to be fixed and adapted to darkness; the subject had to be willing to co-operate and to be trained and tested for reliability. Difficulties were caused by day-to-day variation of response and by the phenomenon of 'subjective light'. There was not a sharp transition from visibility to invisibility as the energy of a light-flash was decreased, and the threshold was defined by an arbitrary statistical measure in the range of critical intensities. His results showed that the human eye could detect flashes of about 100 quanta in the narrow band of blue-green wave-lengths to which the periphery of the retina was most sensitive. Of these 100 quanta in the light-flash, only about 10 reached the retina and were there distributed among about 500 rods.

Discussion of these two papers was divided between questions of methodology and the implications of the empirical observations reported on current theories of perception. There seemed to be little evidence of, or need for, the pure sense-datum required in some theories.

At the final meeting, with Prof. M. H. L. Pryce in the chair, two theoretical physicists discussed the relation between methods of investigation and the characteristics of physical laws. In the first paper, Dr. D. Bohm (Bristol) criticized current views of the role of hypotheses in science. Implicit in Popper's view that scientific hypotheses could only be falsified was the assumption that questions giving answers in the form 'yes' or 'no' could always be put to Nature. But at the boundary of new scientific domains we knew the relevant questions only when the answers were already known. He also criticized the emphasis given to prediction and retrodiction in accounts of scientific method and expressed dismay at the degree of specialization enforced on scientists by the overwhelming mass of data which confronts them. It was important to establish a sound natural philosophy if the full potential of scientific method was to be realized.

The second paper, by Dr. P. W. Higgs (University College, London), reviewed the present untidy field of particle physics and discussed some of the implicit assumptions in this field that theoretical physicists were beginning to question. In particular, the effect of the gravitational field, hitherto regarded as negligible in strength compared with the other fields of particle interaction, seemed to deserve more careful exploration. In the discussion the main points were about the nature of the possible alterna-

tives to prediction and retrodiction as tests of the 'fitness' of hypotheses. What forms could 'diction' take? In what sense were the micro-particles of physics to be regarded as 'ultimate' or 'fundamental'? What exactly was meant by saying that one science or one branch of a science was 'reducible' to another? These were some of the many questions raised and left not wholly answered.

The Vice-Chancellor of the University of Bristol kindly invited the members to a sherry party at Wills Hall on the Saturday evening. Thereafter, small informal groups, oblivious to everything except their own intense discussion, seemed to be generated spontaneously in all the many pleasant corners of Wills Hall and its gardens. At the conclusion of a stimulating week-end, expressions of thanks to the hosts at the University of Bristol, to the staff of Wills Hall and to the organizing secretary were more than mere formalities.

B. C. Brookes

THE NATIONAL RESEARCH COUNCIL OF CANADA

THE forty-third annual report of the National Research Council of Canada, covering the year 1959–60, includes besides the report of the president, Dr. E. W. R. Steacie, of the nine divisions, two regional laboratories and the National Aeronautical Establishment and the financial statement, the annual report of Canadian Patents and Development, Ltd.*.

During the year the Council provided 8·7 million dollars in support of pure research in the universities, compared with 6·3 million dollars in 1958–59, of which 84 per cent was for 875 research grants and the remainder for 425 postgraduate scholarships, fellowships and associateships. The Council's scientific research staff of 658 included 137 postdoctorate Fellows, and in addition 916 technical personnel and 866 general service and administrative staff were employed. Claiming that neither the United States nor the United Kingdom possesses the clear but flexible delineation of administrative authority for science that exists in Canada, Dr. Steacie suggests that in Canada the responsibilities of the Minister for Science are constitutionally exercised by the chairman of the Privy Council Committee on Scientific and Industrial Research, and that in this respect Canada is forty years ahead of the United Kingdom. The problem in Canada is to ensure that scientific activities are so organized as to encourage the full development of the scientific and technical resources of the country. The first responsibility of a national science policy must be support of research in the universities, and the problem is how to maintain the strength and depth of the university science departments and, at the same time, satisfy the requirements of defence, industry and development of national resources with the vigour and competence demanded by international competition, without over-emphasizing technology at the expense of other activities. Dr. Steacie, noting that the Research Council has proved in the past to be an effective channel for supporting science in the universities, urges the need for a continual review of the support of science in the universities to ensure that procedures continue to evolve with the growth of science and changes in administrative practice. Further, while in Canada those departments of Government with administrative responsibilities for natural resources are responsible for the basic research to support their programmes, the Research Council has the function of ensuring the general adequacy of all remaining basic research, but Dr. Steacie stresses the importance of backing Canadian industry by an industrial research effort as great as the scientific resources of the country can support.

Of the work of the Division of Applied Biology, the report notes a co-operative project with the Department of Agriculture on the design and performance of jacketed cold-storage rooms requiring fundamental knowledge of the factors determining low-velocity airflow through shallow ducts, and a study by the Biometrics Section of agricultural meteorology as an extension of the statistical study of protein variability in wheat and wheat exports. Preparations were begun, including design of equipment, for an international expedition to study adaptation to cold by Eskimos. The work of the Division of Applied Chemistry on metals has gradually expanded during recent years, and now includes investigations of the effect of composition and structure on the optical and magnetic properties of a wide variety of alloys. A new technique, developed by the Division about eight years ago for the contacting of fluids and solids, has now been put into commercial operation in grain drying. The Division of Pure Chemistry has shown that ionization potentials of radicals prepared by pyrolytic reactions can be measured by electron impact in the mass spectrometer, and a study of the diffusion of chloride ions in single crystals of sodium chloride and of potassium chloride has shown that the diffusion is very sensitive to the presence of gross imperfections such as dislocations and grain boundaries in the crystals, while attempts to correlate the accumulated observations in the photo-oxidation of ketones have led to the conclusion that not only the rate but also the nature of the products of the reactions of methyl radicals with oxygen depend on the total pressure in the system as well as the presence of molecules containing easily abstractable hydrogen.

In the Division of Applied Physics a new group has been established for plasma research, work was completed in the interferometry laboratory which contributed to an international agreement to base the unit of length, the international metre, on a particular wave-length of the spectrum of an isotope of krypton, and the experiment to determine g, the acceleration due to gravity, by direct observations on a falling body was completed. The group in the Division of Pure Physics concerned with cosmic ray work is still devoting much of its efforts to international co-operation of the type started during the International Geophysical Year, and the development of a space research programme in Canada has permitted cosmic ray measurements with rockets flown to high altitudes. In the X-ray diffraction laboratory

* Forty-third Annual Report of the National Research Council of Canada, 1959–60, including the Annual Report of Canadian Patents and Development, Limited. (N.R.C. No. 5665.) Pp. 40. (Ottawa: Queen's Printer, 1960.)

important results are being obtained in the crystal chemistry of vanadium minerals and the stereochemistry and absolute configuration are being established for certain narcotics the structural formulæ of which have long been in dispute.

The Division of Mechanical Engineering records increasing emphasis on longer term investigations in thermodynamics and engine evolution, and many new propulsion problems in air transport are also being studied, especially those arising from vertical take-off and landing, while certain fluid mechanical aspects of nuclear reactors are being examined. At the National Aeronautical Establishment a substantial research effort has been redirected to problems of high-speed and very high-speed flight, and in particular an increasing effort is being applied to problems arising from very high temperatures. Much of the work of the Radio and Electrical Engineering Division was concerned with defence projects, but the remainder of the programme was concerned with fundamental problems in electrical engineering and electronics, and a pressure gauge capable of measuring the atmospheric pressure at satellite altitudes was developed.

Work at the Prairie Regional Laboratory, Saskatoon, is devoted to a more thorough understanding of the plants and micro-organisms growing on the prairies; but to promote the development of oil-seed crops as alternatives to cereal crops an intensive study of the chemical structure of glycerides was undertaken. At the Atlantic Regional Laboratory, Halifax, where the most efficient way of drying the commercially important plant materials of the region has been studied, a semi-commercial scale batch dryer was developed and a continuous dryer is now being designed and constructed in co-operation with industry.

SAFETY OF YOUNG PEOPLE IN FACTORIES

PROBABLY the most important chapter in the annual report of the Chief Inspector of Factories for 1959* is one of two chapters dealing with subjects of interest without being necessarily restricted to the events of the year—that dealing with the prevention of accidents to young persons. While the consistent downward trend in the number of fatal accidents continued in 1959, there was an increase in the number of reported accidents, and the Chief Inspector is disturbed at the increase in the number of accidents to young persons, from 9,838 in 1958 to 11,001 in 1959. This rise is not due just to an increase in the numbers employed, and indicates clearly that redoubled efforts will be necessary to hold the rate of accidents to young people in check, as increasing numbers of school-leavers enter industry in the coming years. The present drive to train young persons in safe working must be intensified, he writes, not only by industry but also by secondary and technical schools and colleges.

The Chief Inspector, in his letter of presentation, points out that it is evident from the large number of accidents to the head and neck, to the feet and ankles and to the hands, that much more could be done to reduce such accidents by the wearing of suitable clothing, such as safety helmets, goggles, adequate footwear and gloves, and that careful attention would reduce dramatically the grand total of all reported industrial accidents. Young persons, particularly during the first year of employment, tend not to take adequate safety precautions and are more liable to run the risk of suffering injury than older workers. Ignorance of factory conditions and hazards, inexperience and natural curiosity, the desire to prove oneself, contempt for 'safety first' principles, liability to fatigue, and boredom leading to 'skylarking', can all be contributory factors, and there are many examples in the records of the factory inspectorate of the kind of accident in which youngsters are concerned for such reasons.

Young persons, it is clear, enter employment with a handicap, and it is the responsibility of their employers to help them overcome it by providing sound training and careful supervision and by insisting on high standards of obedience to safety rules. Many of the large firms have, in fact, built up excellent organizations for this purpose, and in his annual report for 1951 the Chief Inspector, Sir George Barnett, attributed the considerable reduction in the post-war accident-rate mainly to this factor. Many managements, however, did not trouble to make allowances, and since then the rate of decrease of accidents to young persons has slowed down, and the decrease in the number of accidents to young persons is, moreover, proportionately less than the corresponding decrease for all employed persons.

While there have been several important pieces of legislation since 1937 affecting young persons employed in factories, such as prohibition or restriction of employment in certain dangerous or unhealthy trades, prohibition of employment on certain types of dangerous machinery or in carrying or lifting excessive weights, limitation of the number of hours a young person may work and the provision of medical supervision, the legal contribution must be supplemented by effective training and supervision, beginning in schools and colleges and continuing in industry. There are few places where the young can be prepared for work in the factory so well as in secondary school and college workshops and housecraft rooms. Classes there provide an excellent opportunity for showing how tools should be maintained and used, and machines properly guarded. The teacher, too, can inculcate a degree of safety consciousness and an acceptance of discipline in the correct use of machines and tools which is invaluable in factory life. As the Chief Inspector observed in addressing a conference on "Accident Prevention in Technical Colleges" at Blackpool in June 1958, arranged by the North West Regional Advisory Council for Further Education, his teachers are the best people to give the neophyte entering the factory the confidence that he requires.

The Chief Inspector did not suggest that safety should be taught as a subject *per se*, but rather that safety teaching should go hand in hand with the various subjects, and in the present report the Chief

* Ministry of Labour. Annual Report of the Chief Inspector of Factories on Industrial Health, 1959. Pp. iii+60. (Cmnd. 1137.) (London: H.M. Stationery Office.) 3*s*. 6*d*. net.

Inspector refers to encouraging developments as a result of the initiative of the North West Regional Council. The Council itself set up an Industrial Hazards Sub-committee, which organized three courses in accident prevention at Liverpool, Manchester and Preston, and afterwards the Cheshire County Education Authority established a permanent committee to deal with safety training in colleges in Cheshire, on which both industry and college staffs are represented. The general problem has also been discussed at a conference held recently in Glasgow, attended by a number of principals, heads of departments and teachers from technical colleges in Scotland, and addressed by senior members of the factory inspectorate.

Among the suggestions which found favour with the conference were publication of a safety code for each college; appointment of a member of the teaching staff to be specially responsible for safety matters; inclusion in examination papers of questions relating to safety; and close liaison between the technical colleges and inspectors of factories, who can give occasional lectures and advise on safety precautions. A pamphlet, comprising mainly speeches delivered at the Blackpool conference, has already been distributed to technical college teachers in the north-west region and received a wide circulation in other parts of Britain, but these efforts are only a beginning and, as the Chief Inspector now emphasizes, much more remains to be done if young people are to come into industry adequately prepared and the transition from school to industry is to be as smooth as possible. He urges that it is important for the success of this work that education authorities and individual principals and heads of departments at technical colleges should recognize the seriousness of the problem and the need to take steps to deal with it.

It might be added that this is a matter that could well be recognized as a professional responsibility by scientists and technologists, not simply when their own work as teachers or in industry brings them in direct contact with it, but through their professional organizations. Their co-operation and guidance can sometimes make the decisive difference between success and failure, and this is no less true in respect of some of the suggestions for action by employers which are made in this special chapter of the Chief Inspector's report. Apart from care in picking young people for the work they are going to do, training schemes should include six particular points. The young worker should be taken around those parts of the factory where he will work and should be shown what the principal dangers are, how they arise and how to avoid them. A fully competent adult worker should directly supervise the young person's work during the initial period of training and the proper method of working any machine on which the youngster is to be employed should be illustrated in detail and the dangers associated with other undesirable methods explained. Departmental managers and other supervisory grades should be given clear instructions on accident prevention and on the need for keeping close control over the work of the young persons under their charge, and strict discipline should be enforced in the adoption of safe methods. Special safety propaganda should hammer home the important points and make young workers aware of the importance which is attached by the employer, and all in supervisory positions, to the principles of accident prevention.

Clearly not all these points concern either the scientist or technologist as such, but in many of them his interest and co-operation can be decisive factors. Moreover, as the report concludes, it is not only firms with large numbers of youngsters and great financial resources that can achieve substantial results. It is well within the capacity of managers and supervisory staffs in smaller firms to make the effort to see that their young recruits have a good and safe start in their chosen career, and many such managers and members of supervisory staffs, as well as teachers and departmental heads in technical colleges, are themselves likely to possess scientific or technological qualifications.

THE COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION IN AUSTRALIA

THE eleventh annual report of the Commonwealth Scientific and Industrial Research Organization in Australia, covering the year 1958–59*, notes a number of changes in organization.

The Division of Industrial Chemistry has become the Chemical Research Laboratories, with three Divisions, Chemical Physics, Mineral Chemistry and Physical Chemistry, and three independent Sections, namely, Cement and Ceramics, Chemical Engineering and Organic Chemistry. Similarly, the Wool Textile Research Laboratories have been renamed the Wool Research Laboratories, with Divisions of Protein Chemistry, Textile Physics and Textile Industry. The Soil Mechanics Section of the Division of Soils has become an independent section of the Organization and the unit of the Division of Plant Industry and Plant and Soil Laboratory, Brisbane, becomes an independent Division of Tropical Pastures, while the former Radio Research Board Laboratory, Camden, New South Wales, has become an independent Upper Atmosphere Section. Collaboration with the universities has been further extended by the establishment with the University of Melbourne of a joint laboratory for research and soil mechanics at Syndal, Victoria, and of a Meat Research and Teaching Centre at the University of Sydney Animal Husbandry Farms, Camden, New South Wales. A Rice Research Station is being established on the coastal plains near Darwin, where studies will be made of nutrition and water requirements of rice and of possibilities for developing varieties well adapted to that environment. Two frigates, specially equipped for oceanographic survey work, are being made available to the Organization for periods of about 12 weeks a year, and this assistance will facilitate the more comprehensive and regular programme of observations which must be undertaken before a clear picture of the oceanography of the waters surrounding Australia can be developed.

* Eleventh Annual Report of the Commonwealth Scientific and Industrial Research Organization for year 1958–59. Pp. 181. (Canberra: Commonwealth Government Printer, 1960.)

In co-operative industrial research support of the Australian Leather Research Association, the Bread Research Institute of Australia, the Wine Research Institute and the Australian Coal Association (Research), Ltd., continued. New research projects sponsored by industry commenced during the year included investigation of solid lubricating materials derived from Australian beach sand minerals; development of methods for determining the oxygen content of cyanide solutions used in extracting gold from its ores; research on the colorimetric properties of pigmented materials; and the study of changes in shell egg quality during marketing. The design study for the proposed giant radio-telescope for the Division of Radiophysics has been completed, and the radio telescope is scheduled for completion early in 1961.

During the year 24 junior and 26 senior postgraduate studentships were awarded as well as 14 overseas research studentships. Of the Organization's income of £8,060,831 in 1958–59, £6,297,597 was from Treasury appropriation and £1,322,576 from the Wool Research Trust Fund Account. Of the expenditure of £7,538,477 on research activities, £776,186 was on animal health and production, £831,237 on the plant industry, £263,480 on food preservation and transport, £336,868 on forest products, £678,571 on chemical research, £198,144 on fisheries and oceanography, £657,310 on the National Standards Laboratory, £157,049 on building research, £403,453 on radiophysics, £446,402 on wool research, £203,276 on fuel research, £125,962 on wildlife survey, and £202,226 on land research and regional survey. Besides summarizing the work of the Organization during the year in nearly thirty different fields, the report includes details of the membership of the Council and its committees and of the professional staff of the Organization as well as a list of papers published during the year.

THE INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY

The Physical Society

AT the extraordinary general meeting of the Physical Society held on July 5, in London, the reports of the council and the accounts for the year ended December 31, 1959, were presented and adopted and the special resolutions for the voluntary winding up and the transfer of the assets of the Society to the newly amalgamated body, the Institute of Physics and the Physical Society, were unanimously carried.

The year under review in the annual report is stated to have been very satisfactory from the financial point of view, with an overall excess of income over expenditure of £3,796. The Exhibition Contingency Fund was increased by £4,000 to £12,000, but the exhibition account still showed a profit of £1,966. The 1959 exhibition of scientific instruments and apparatus was held earlier in the year, January instead of March, but there was no falling off in the keenness of exhibitors to obtain space or in the attendance of visitors to the exhibition.

The "Handbook of Scientific Instruments and Apparatus", the catalogue of the exhibition, was in great demand and sold well. The flow of manuscripts for publication in the Society's *Proceedings* remained steady during 1959, but because of the printers' strike the volume of material published was less than in former years. 174 papers, 72 research notes and 25 letters to the editor were published, and 54 out of a total of 302 manuscripts received were rejected. Volume 22 of the "Reports on Progress in Physics", which was published during the autumn, was the largest for some years and contained fourteen articles. The sale of past issues of both *Proceedings* and the "Reports", as well as of reprints of individual articles in the "Reports", showed a marked increase. A list of the various lectureships, prizes and medals awarded by the Society during 1959 and their recipients is given in the annual report.

The lectures and the presidential address are published in the "1959 Year Book of the Physical Society". Brief reference is also made in the report to the activities of the Society during the year and in particular to the conferences lasting two or three days which were held on various subjects in Birmingham, Durham, Edinburgh, London and Oxford. The attendances were usually between 100 and 300, with members of the Society forming the majority.

The Institute of Physics

An extraordinary general meeting of the Institute of Physics was held on July 5, 1960, in the Institute's House, 47 Belgrave Square, London, with the president, Sir George Thomson, in the chair. The first part of the meeting took the form of the annual general meeting and the fortieth annual report of the Board of the Institute of Physics was presented and adopted*. The meeting then considered three special resolutions concerned with the winding up of the Institute and the handing over of its property and its funds to the amalgamated body, the Institute of Physics and the Physical Society. The resolutions were carried unanimously.

The report records the continued growth of the Institute, both in the number of its members and in its activities in many fields, including education, scientific publications and conferences, symposia and meetings arranged by its branches and specialist groups. During the year, 933 applications for election or transfer to the various grades of membership were received, and the total membership increased by 445 to 6,754. Five technical colleges applied for recognition by the Institute as institutions having courses of work approved for the purpose of the membership regulations and all five were accepted.

Thirty-nine of the 119 candidates who presented themselves for the examination for the graduateship grade of membership were successful; 13 possessed university degrees and 26 the Higher National Certificate in applied physics. The fifteen question papers, together with the examiners' comments, were published in the September issue of the *Bulletin* of the Institute, and afterwards also as a pamphlet.

* Institute of Physics. Fortieth Annual Report of the Board of the Institute of Physics for the year 1959. Pp. 19. (London: Institute of Physics.)

New regulations and syllabuses for the graduateship examination, which will be in two parts, are to come into operation in 1962. There were 820 candidates from 46 technical colleges for the Ordinary National Certificate in applied physics and 296 from 23 colleges for the Higher Certificate.

A new edition of the booklet "The Scientific Education of Physicists", which gives guidance on training and a summary of information about the physics departments of all universities and recognized technical colleges in Great Britain, was published during the year. A symposium on "Current Developments in the Production of High Vacua" was held in London on April 17, and a two-day conference on "Some Aspects of Magnetism" in the University of Sheffield during September 22–24. Summarized accounts of the proceedings of the symposium and of the conference have been published in the *British Journal of Applied Physics*. Books published for the Institute included reprints of the first five monographs in the "Monographs for Students" series, and a new volume, "Mechanical Design of Laboratory Apparatus", by H. J. J. Braddick; "The Design of Physics Research Laboratories", the proceedings of a symposium arranged by the London and Home Counties Branch of the Institute; and a second edition of "Physics as a Career".

Details are given in the annual report of the amount of material submitted and published during the year in the Institute's two monthly journals, the *British Journal of Applied Physics* and the *Journal of Scientific Instruments*. The Journals Advisory Committee at its meeting, held during May, agreed that the scope of the *British Journal of Applied Physics* should not be limited despite the appearance of several new journals devoted to particular branches of applied physics. The circulation of the *Journal* continued to increase, but that of the *Journal of Scientific Instruments* showed a slight decrease for the first time for many years. The page size of the *Bulletin* was increased to conform with that of the two *Journals*, and the 324 pages of text, which contained about 22 per cent more material than the 336 pages of the 1958 volume, included twenty-five articles and 114 book reviews, in addition to Institute news.

The activities of the Australian, Malayan and the nine Great Britain branches, together with those of the specialist groups of the Institute, are outlined separately in the report. Two conferences were held by the Australian branch in Melbourne, the second "Conference on Contemporary Optics" during February 25–27, and the "Solid State Physics Conference" during August 17–21. A new division was formed in the Australian Capital Territory. The Applied Spectroscopy Group held an all-day symposium on "Reviews of Recent Developments in Spectroscopy" in May; the Education Group an Easter conference on the teaching of science to children up to the age of fifteen; the Electronics Group a two-day meeting in October on the subject of noise in electronic devices; and the Non-destructive Testing Group the first joint conference with the Société Française de Métallurgie in Paris. The Stress Analysis Group undertook a new venture in April when it arranged a three-day international conference on stress analysis in Delft, Holland. Fifty-one papers covering a wide range of topics were presented, and a selection of the papers is to be published in book form.

The annual dinner of the Institute was held on May 5, at the Park Lane Hotel, London, when 265 members and guests were present. The speakers were the president, Sir George Thomson, Sir Keith Murray, Sir Harry Melville and Mr. O. W. Humphreys. The alterations to the Institute's House, which had to be postponed for reasons of economy, were completed during 1959, and the senior staff dining room which had been used temporarily as an office was converted into a committee/dining room. The furnishings and décor were provided for by friends of the Institute and from the bequest of Mrs. W. Phillips. The Institute's accounts show a substantial increase in members' fees and on the publication business. £3,450 was transferred from the Institute's accounts to the Endowment Trust Fund account, but this was only just sufficient to cover the cost of the scientific and educational activities carried out by the Fund for the Institute. The Benevolent Fund continues to be well supported by members, and a further donation of £A.200 received from the Australian Branch's Special Fund and an additional payment of £258 in respect of tax recovered on payments made by the Trust set up under the will of the late Mrs. W. Phillips were very welcome. During the year £936 was paid out in assistance to ten cases.

FERAL MUNTJAC DEER (*MUNTIACUS* SPP.) IN THE WEST MIDLANDS

TWO species of muntjac deer, the Chinese (*Muntiacus reevesi*) and the Indian (*M. muntjac*), were introduced into England at Woburn Park, Bedfordshire, about the beginning of this century. Later they were kept at Whipsnade and at least two private zoos (Crowland Abbey, near Wellingborough, and at Broxbourne, Herts). During the past half-century, muntjac have been reported in Bedfordshire, Northants, Warwickshire, Bucks, Herts, Berks, Cambridgeshire, Huntingdonshire, Essex, Leicestershire, Middlesex, Norfolk, Oxon., Staffordshire and Suffolk. Only in the last five counties can it be stated as certain that the deer are established as a regularly breeding species at the present time.

The successful colonization by the muntjac of a wide area of Britain has taken place very largely without the knowledge of its human inhabitants. T. J. Pickvance and J. S. R. Chard have prepared an article directing the attention of naturalists and others to the presence of this small deer and indicating some of the problems involved in recording the distribution and habits of the muntjac (*Proceedings of the Birmingham Natural History and Philosophical Society*, 19, Part 1; 1960).

The muntjac is unlikely to become the object of measures of extermination. Unlike the larger species of deer, it does no harm to trees, and from places where it sometimes becomes a nuisance, such as market

gardens and allotments, it can be excluded by wire netting. Control will not prove difficult as it is easily caught, and, being a creature of habit in its movements, is not difficult to shoot. Its small size and retiring habits aid its unobtrusive spread. Thus, an increasing number of naturalists will be able to study this species which has an interesting form of territorial behaviour.

The main part of the article deals with records of the colonization by the muntjac of Warwickshire. These are typical of those gathered during surveys made with the view of determining not only the present distribution of the animal but also the rate and direction of colonization and the past history of the movements which have taken place.

NOMENCLATURE OF THE NICOTINAMIDE NUCLEOTIDE COENZYMES

By Dr. MALCOLM DIXON, F.R.S.
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THE nomenclature of the nicotinamide nucleotide coenzymes has been a subject of long-standing disagreement, and unanimity has not yet been reached. Several different systems are in use to varying extents.

Included with other subjects in the terms of reference of the Enzyme Commission of the International Union of Biochemistry[1] is the nomenclature of enzymes and of coenzymes, and during the past three years the Commission has been giving careful and detailed consideration to the question of coenzyme nomenclature in an effort to reach a generally acceptable solution. The question has also been considered by the Biological Chemistry Nomenclature Commission of the International Union of Pure and Applied Chemistry. A summary of the points involved and the reasons for the recommendations of the Commissions may be of interest.

Present Position

The situation that has to be faced is that, except perhaps in the United States, there is no unanimity about the naming of the two coenzymes. Four different systems are in use, although to very different extents, and the special difficulty of the position arises from the fact that the system that is most used is that which has the strongest objections from the chemical point of view. The four systems are: (*a*) cozymase and phospho-cozymase; (*b*) codehydrogenase I and codehydrogenase II (or codehydrase I and codehydrase II by some Continental writers); (*c*) coenzyme I and coenzyme II (abbreviated to CoI and CoII); (*d*) diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN). The first system is probably the least, and the fourth the most, used at the present time. None of these systems is satisfactory, the first three because they are uninformative, the last because it is incorrect, as a number of reviewers have pointed out[2–5].

There is rather more consistency among the journals than there is among authors of books and reviews. The *Journal of Biological Chemistry*, which had previously used both cozymase and coenzyme I, first used diphosphopyridine nucleotide in about 1940; and it has continued to do so, along with cozymase and coenzyme I, until about 1950, and as the sole form after that date. The *Biochemical Journal* used cozymase and coenzyme I, and did not permit diphosphopyridine nucleotide, until as recently as 1953, but since then has used the last name almost entirely. The index of *Chemical Abstracts*, however, still uses codehydrogenase I rather than DPN.

Di- and Tri-phosphopyridine Nucleotide

There would be no objection whatever to the use of the names diphosphopyridine nucleotide and triphosphopyridine nucleotide if they did not indicate chemical structures, but the main objection is, in brief, that not only do they fail to give the structure of the coenzymes properly but also that they are the chemical names of other compounds. It is somewhat like using the name 'methyl acetate' for pyruvate; the name indicates a structure, but it is the wrong structure.

The names are derived from, though not the same as, names introduced by Warburg[6] in 1936 as convenient descriptions. The term 'pyridine nucleotide' was used, quite legitimately, to distinguish this class of compounds from purine nucleotides such as the adenine nucleotides. To distinguish the coenzymes from each other they were described as 'the diphospho-' and 'the triphospho-pyridine-nucleotide', at first with the definite article, although this was almost immediately dropped and the terms thereafter used as names in the German forms 'Diphospho-Pyridinnucleotid' and 'Triphospho-Pyridinnucleotid'.

'*Diphospho-*'. In 1939, F. G. Fischer[2], in his review on the subject, pointed out that these names, though concise, were incorrect. According to chemical terminology, 'phospho-*X*' denotes *X* combined with an additional phosphate group; but the word 'nucleotide' already includes a phosphate group, thus a 'diphospho-(di)nucleotide' would contain four phosphate groups, and not two as does the coenzyme. Fischer suggested that, especially in relation to the accepted names of the analogous flavin nucleotides, the names 'Pyridine-Adenine-Dinucleotide' and 'Phospho-Pyridine-Adenine-Dinucleotide' would be clearer. They would have the advantage of showing that the compounds are dinucleotides containing adenine, which was not indicated by the earlier names. Probably because of the outbreak of war in the same year, these suggestions did not receive the attention they deserved.

'*Triphospho-*'. It was at first believed that the three phosphate groups of triphosphopyridine nucleotide were joined in line, as in adenosine triphosphate, so that the relationship between the two coenzymes would be analogous to that between adenosine diphosphate and adenosine triphosphate. When the true structure of triphosphopyridine nucleotide was determined by 1950 by Kornberg and Pricer[7], it was found to be a monophospho-dinucleotide, so that the prefix 'triphospho-' became less appropriate.

'*Diphosphopyridine-*'. Perhaps the most serious objection arose when the names became anglicized,

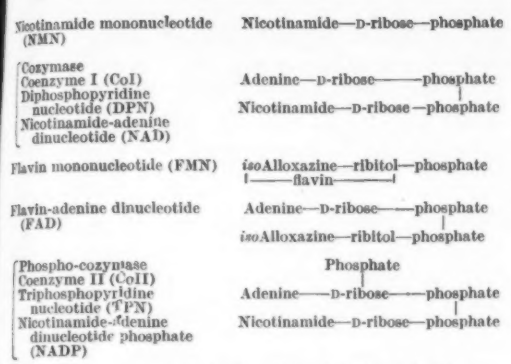

Fig. 1. Structures of nicotinamide and flavin nucleotides

first I believe in about 1940 in the United States. The form used by Warburg, namely, 'Diphospho-Pyridinnucleotid', was clearly intended to be taken in the sense of diphospho(pyridinenucleotide), as shown by the hyphen and the capital P. The introduction of a space before 'nucleotide' gave a form with quite a different meaning. The name 'diphosphopyridine nucleotide' means quite unambiguously a nucleotide of diphosphopyridine, that is, a substance of the structure diphosphopyridine—D-ribose—phosphate, and it is not surprising that the biochemists have often been criticized by the organic chemists for using such names.

'*Dihydrodiphospho-*'. For the reduced or dihydro-forms, diphosphopyridine nucleotide and triphosphopyridine nucleotide yield names beginning with the undesirable prefix 'dihydrodiphospho-', which is a further objection.

'*Pyridine-*'. One may properly use the term 'purine nucleotides' for the whole class, but in referring to a particular nucleotide the name of the particular purine is used. One says 'flavin-adenine dinucleotide' and not 'flavin-purine dinucleotide'. Correspondingly the word 'pyridine' ought not to be used for the names of particular nucleotides, unless they are in fact nucleotides of pyridine itself. 'Pyridine' should not be used when 'nicotinamide' is meant. The coenzymes are nicotinamide compounds, and the corresponding pyridine compounds are inactive as coenzymes[8]. The diphosphopyridine nucleotide system of nomenclature causes great difficulty in the rapidly expanding work on analogues of the coenzymes. If the nicotinamide nucleotides are called 'pyridine nucleotides', what can one call the pyridine nucleotides ? They surely cannot be called pyridine analogues of the pyridine nucleotides ! The use of diphosphopyridine nucleotide forces the use in current literature of such terms as 'pyridine-DPN' and 'APDPN' (for acetyl-pyridine diphosphopyridine nucleotide), in which the word 'pyridine' occurs twice, although there is only one pyridine ring in the compound. This is a very real difficulty.

'*DNP*'. DNP is the accepted abbreviation for dinitrophenol, a reagent much used in work on the coupling of biological oxidation and phosphorylation. Papers on DNP often contain many references to DPN, and the similarity makes them confusing and difficult to read.

'*FMN, FAD, NMN, — ?*'. Finally, the name diphosphopyridine nucleotide obscures the close and important analogy with the corresponding flavin compounds, shown in Fig. 1. Flavin-adenine dinucleotide (FAD) is the same substance with the nicotinamide end changed into the flavin structure. Flavin mononucleotide (FMN) is the same without the adenylic acid half of the molecule. Nicotinamide mononucleotide (NMN) is the coenzyme without the adenylic acid. These three names and the corresponding abbreviations are quite generally accepted, and there is no suggestion that they should be changed. To complete the scheme we should expect NAD for nicotinamide-adenine dinucleotide, thus :

FMN	NMN
FAD	NAD

The use of DPN instead of NAD breaks the logic of the nomenclature.

Summary of Objections

To recapitulate, the main objections to the name diphosphopyridine nucleotide are : (*a*) the coenzyme is not a nucleotide of diphosphopyridine as the name indicates ; (*b*) the name indicates the presence of four phosphate groups instead of two ; (*c*) it does not indicate that the coenzyme is a dinucleotide or that it contains adenine ; (*d*) the coenzyme is not a nucleotide of pyridine but of nicotinamide ; (*e*) the pyridine nucleotides, to which the name properly applies, are inactive as coenzymes ; (*f*) the use of the name for the nicotinamide compound makes it impossible to name the pyridine analogues satisfactorily ; (*g*) the combination 'dihydrodiphospho-' in the name of the reduced form is undesirable ; (*h*) the name is out of line with the FMN FAD NMN sequence and obscures the close chemical analogy with the flavin compounds ; (*i*) the corresponding name triphosphopyridine nucleotide suggests a triphosphate structure rather than the actual monophospho-dinucleotide structure.

In view of the strong chemical objections, the Enzyme Commission felt itself unable to recommend the continued use of the DPN system of nomenclature, particularly as a satisfactory alternative was available. The difficulty is not a chemical one, for there is no doubt about what the substance is : it is nicotinamide-adenine dinucleotide. It is the psychological difficulty of bringing the correct name into use, when an incorrect name has become well established. The Commission fully realizes that it is taking a serious step in recommending a change, and that there is likely to be some opposition, perhaps especially in America, as there was in Britain when DPN replaced Co I. However, this moment, when a real effort is being made to bring the terminology of enzymology into order, is the right one to put the coenzyme nomenclature right also. It is probably the last opportunity there will be of doing so ; if it is not done now, there is not likely to be another chance.

Some workers feel that the whole matter should be left optional, so that authors should be free to use any system they wish. The difficulty facing the Commission, however, was that, apart from the fact that it was given the task of dealing with coenzyme nomenclature, it also had to deal with the nomenclature of enzymes, and the names of a large number of enzymes depend on those of the coenzymes. It was therefore obliged to make a choice, in order to avoid the necessity for more than one systematic name for each of these enzymes.

Nicotinamide-adenine Dinucleotide

It has therefore decided to recommend that both Co I and DPN should be dropped, and that the

coenzyme should be known by its actual chemical name, nicotinamide-adenine dinucleotide, which may be abbreviated to NAD. It will be seen that this name avoids all the objections mentioned above, it indicates the structure of the compound, it brings out the analogy with FAD, and it makes it possible to name the analogues satisfactorily. The reduced form would be named dihydronicotinamide-adenine dinucleotide, which indicates correctly the part of the molecule that becomes reduced.

Nicotinamide-adenine Dinucleotide Phosphate

Triphosphopyridine nucleotide (CoII) might be named phospho-nicotinamide-adenine dinucleotide, but this is undesirable, for 'phospho-nicotinamide' is as incorrect as 'diphosphopyridine', and the reduced form would again have the 'dihydrophospho-' prefix. The recommended form 'nicotinamide-adenine dinucleotide phosphate' (NADP) avoids both these objections, and the extra P clearly indicates the formation from NAD by the addition of a phosphate group.

Coenzyme Analogues

Using the NAD system, the naming of the analogues becomes relatively simple and straightforward. For example, the pyridine analogue would be called pyridine-adenine dinucleotide, which might be abbreviated to PAD if desired. The deaminated form is at present called 'desamino-diphosphopyridine nucleotide', but this gives little idea of its nature, since the part of the molecule which is deaminated (the adenine) is not mentioned in the name. The obvious name for it is nicotinamide-hypoxanthine dinucleotide (NHD). The analogue of TPN in which the phospho-group is attached to the 3′-position instead of the normal 2′-position is at present called '3′-triphosphopyridine nucleotide', which surely cannot be taken as indicating only one phosphate group attached to the 3′-position. The new system would give the natural name nicotinamide-adenine dinucleotide 3′-phosphate, perhaps abbreviated to NAD3P. Other analogues should cause no great difficulty. Abbreviations for the analogues are not being officially suggested; these are merely given as personal suggestions to illustrate the great advantages of the NAD system.

Possible Objections

It remains to consider whether the names now proposed for the coenzymes are in strict accordance with chemical terminology.

'*Nucleotide*'. Can nicotinamide mononucleotide and flavin mononucleotide strictly be termed nucleotides? If the definition of a nucleotide is restricted to substances that can be obtained from nucleic acids (that is, to purine and pyrimidine nucleotides), they cannot, because of the different bases present. Probably, however, nobody would wish to narrow the definition in this way, and it is much more reasonable to use the term to denote the chemical structure: base—pentose—phosphate. Clearly nicotinamide mononucleotide qualifies as a nucleotide.

The case of flavin mononucleotide is not so clear, for two reasons. In the first place, it contains not ribose but ribitol, so that the —CHOH— group in position 1 of the ribose is represented by a —CH_2— group in the flavin. However, the term nucleotide is also applied to the deoxyribonucleotides, in which the —CHOH— group in position 2 of the ribose is replaced by a —CH_2— group. It is not unreasonable, therefore, to make the term cover both modifications, although the change in the 1-position is the more important, since it prevents ring formation. In actual fact, the flavin compounds have been universally called nucleotides for well over twenty years, and there is no suggestion that their nomenclature should be changed.

In the second place, the term 'flavin' includes not only the base but also the ribitol. Therefore the term 'flavin nucleotide' must be understood as a nucleotide containing flavin, rather than a nucleotide 'of' flavin in the sense in which NMN is a nucleotide of nicotinamide.

'*Dinucleotide*'. Finally, can nicotinamide-adenine dinucleotide and flavin-adenine nucleotide be strictly termed dinucleotides? A purist might hold that the term implies that the two mononucleotides are linked in the same way as are the nucleotides in nucleic acid, by a 3′-5′-linked phosphate group. However, it would seem reasonable to consider a compound formed by the simple union of two mononucleotides to be a dinucleotide, particularly as the two classes of dinucleotides only differ in the point of attachment of a single bond.

'Dipeptide' has been quoted as an analogy where the term implies a definite point of linkage, but the analogy is not a valid one. A dipeptide is not formed by the union of two monopeptides, and the name is clearly based on different principles. A truer analogy would be 'disaccharide', which is used for a compound formed by joining two monosaccharides, without respect to the point of attachment.

Substances of the flavin-adenine nucleotide type have been called dinucleotides ever since their first discovery, and it is the only name available. It has probably appeared far more often in the literature in this sense than in the sense of the 3′-5′-linked compounds, and it would be unreasonable now to restrict its use to the latter type.

Conclusion

Such are the considerations which have led the Enzyme Commission of the International Union of Biochemistry to recommend the use of NAD and NADP. The Biological Chemistry Nomenclature Commission of the International Union of Pure and Applied Chemistry has also decided, after considering the possible alternatives, to recommend these names in place of the existing systems. Preliminary experience has shown that, even those whose first reaction is to express a preference for the retention of DPN, the new names are very quickly found to be attractive and satisfactory. It is hoped that when they become the official recommendations of both International Unions the journals will give a lead in their adoption.

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[2] Fischer, F. G., *Erg. Enzymforsch.*, **8**, 186 (1939).
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[8] Kaplan, N. O., and Ciotti, M. M., *J. Biol. Chem.*, **221**, 823 (1956).

STABILIZATION OF SEDIMENTARY COASTLINES

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Department of Civil Engineering, University of Western Australia

THE major problems in coastal engineering originate from the mobility of sediment on the ocean floor in the vicinity of coastlines. Recent attempts at tracing this movement have involved the use of radioactive isotopes. These are costly, can be a hazard to health and generally suffer from a short life for tracking purposes. Unless material can be identified for at least twelve months, the net motion cannot be accurately ascertained.

Another approach appears simpler and more rewarding. It is based on the premise that the major features of a coastline emanate from centuries of interaction between geological and climatic factors. Of the former, wave action is the most important, particularly the action of swell, although storm sequences play their part by putting the sediment continually back into circulation. But in the end the predominant swell will give the coastline its particular outline, the recognition of which can help define the direction of the net sediment movement.

Model Tests

Tests have been carried out at the University of Western Australia to determine this equilibrium shape for some persistent swell direction. In a scaled model of a typical coastline, waves were generated at an angle of 45° towards a straight length of sandy beach in which were included three concrete sections resembling rocky headlands. Up-coast of the two beach units so formed, no sand replenishment occurred, and the coast was allowed to erode. Material was removed beyond the down-coast rock outcrop so as not to interfere with the natural shaping of the coastline.

The depth of the water in the model was constant at 7 in. and the beach was built up flat 3 in. above this level, with 20 ft. distance between headlands. No scale can be attached to the study as no prototype conditions existed for it; but if an undistorted scale of 1 : 60 were to be placed on the previous dimensions, the waves generated would have represented prototype waves of 10-sec. period and 5-ft. height approximately.

The waves were generated for intervals of several hours, after which a survey was made of the waterline and the underwater depths. This was continued until a quasi-permanent shape resulted in the up-coast unit, which occurred when any wave broke simultaneously around the length of the bay so formed. Fig. 1 shows the progressive stages in the beach-line of the two tests carried out. Fig. 2 shows the underwater contours at the end of each test.

The conclusions that may be drawn from these figures are:

(*a*) The equilibrium shape for a sedimentary coastline with oblique swell is that of a half-heart with the curved portion up-coast and the tangent section down-coast of the bay unit. Although the final shapes obtained in the up-coast bays of the model tests only approximated to this, it can be reasoned that with intermittent storm waves and slight alterations in wave directions the beach-lines would have been smoothed out. (It is intended to conduct such tests in the future.) The straight section should be almost parallel to the crests of the waves in deep water as they are refracted very little, whereas the curved portion of the bay is sculptured by the waves which are diffracted around the up-coast headland and refracted inside the bay.

(*b*) The shape of the down-coast bay at any time is dictated by sediment passing to it from the up-coast bay. In Fig. 1 the former is more eroded in Test No. 1 than Test No. 2 because less material has been removed from the up-coast unit. It can be seen how the supply of sediment to the down-coast bay will vary continuously throughout the full development of the up-coast bay.

(*c*) From the underwater contours of Fig. 2, it is seen that a circular current has developed which has caused a shoal near the tangent point. In Nature this is not likely to be so pronounced because of the intermittent nature of the wave occurrence and the influence of storm sequences. It is this circulation that has helped cause the irregular shape of the model coastlines, because the bottom contours influence the refraction pattern of the waves.

But the principal deduction is that, given a persistent swell from an oblique direction moving sediment down-coast, an erosive situation will cause bays of the half-heart shape to be fashioned between the headlands. Equally, the occurrence of such bays will indicate the net movement of sediment and the direction of the persistent swell.

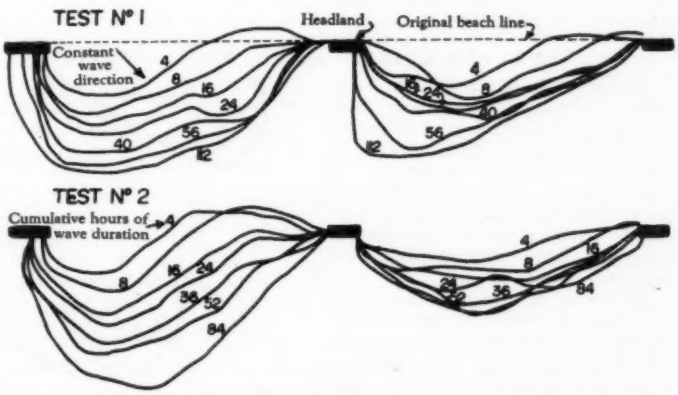

Fig. 1. The model beach-line after specified hours of wave duration from a constant direction

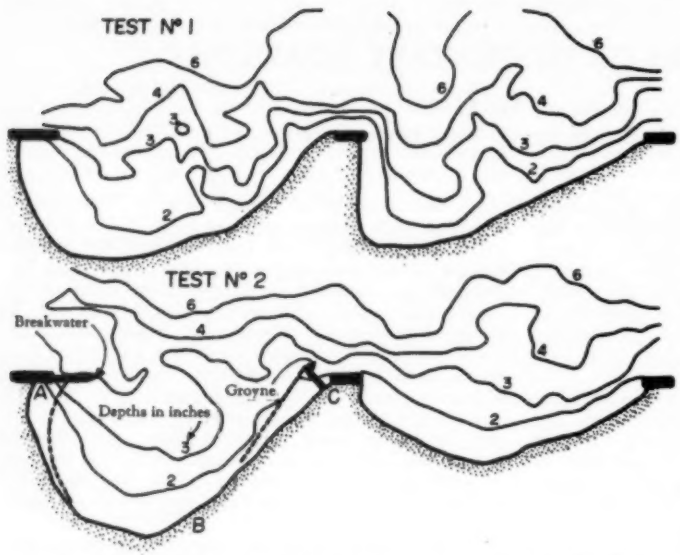

Fig. 2. Underwater contours in the model when the up-coast bay was in quasi-equilibrium. Accreting effect of the groyne and breakwater is shown dotted

Equilibrium Shape in Nature

To check the validity of this contention in Nature, a section of the South African coast has been chosen where a predominant wave direction is known to exist[1,2]. An outline of the coast is sketched in Fig. 3. As indicated, the persistent swell is from the south-west, and the bays have taken the half-heart form of which the tangent section tends to be normal to the wave direction. The shape of the continent would preclude much movement of sediment eastward around Cape Agulhas, and hence erosion has occurred along this section of coast over the centuries and is probably still doing so. Notwithstanding any seasonal or other reversals, the net or long-term movement of sediment is towards the east.

With the acceptance of conditions producing the equilibrium shapes of coastline, it is possible to produce a map showing the net sediment transport around the coastlines of the world. I am at present undertaking this, checking areas with less-pronounced features by referring to any ocean-swell charts which are available.

Stabilization of a Coast

Although reversals of drift can cause problems of erosion and silting in man-made coastal structures, the major difficulties arise from the long-term movement in one direction. Attempts at reducing or prohibiting this motion of sediment, generally by means of groynes, have met with varied success.

Referring to Fig. 2, it is apparent that if groynes are constructed between B and C they are equivalent to extending the headland out to sea and, assuming that there is still some longshore movement, the shoreline will advance by accretion to the new line shown dotted. The groyne here has accomplished its purpose. On the other hand, groynes built in an area AB, if it is still subject to erosion, will be ineffective. Should the bay be almost stable, groynes built there might sustain the coastline against seasonal fluctuations, although it has been shown elsewhere how groynes can accelerate erosion[3]. A more effective means of building up the coast between A and B is to extend the headland at A by means of a breakwater or other structure. The waves then have a new centre of diffraction and will effect a new shape as indicated.

On a coastline where there is substantial net movement of sediment, the best means of reducing it, or preventing it altogether, is to construct headlands about which the stable half-heart bays may form. The distances between these headlands should be of the order of miles, so that a wide expanse of

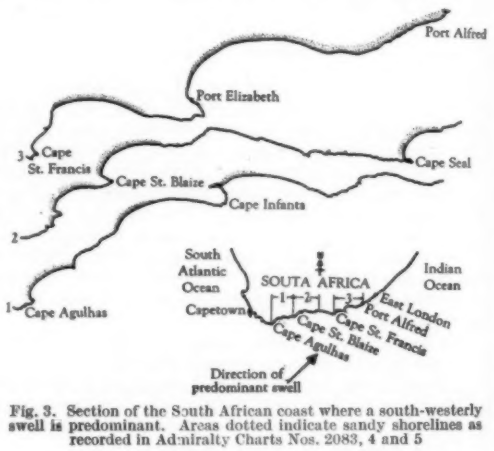

Fig. 3. Section of the South African coast where a south-westerly swell is predominant. Areas dotted indicate sandy shorelines as recorded in Admiralty Charts Nos. 2083, 4 and 5

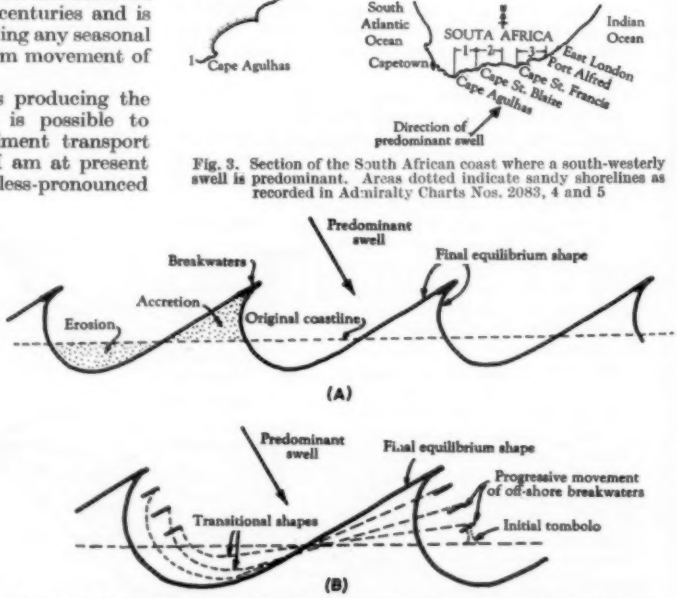

Fig. 4. (*A*) A method of preventing long-term movement of sediment along the coast, showing allowances for erosion and accretion. (*B*) A step method of approaching the stable half-heart shape by means of a movable offshore breakwater

ocean floor is affected. Allowance will therefore have to be made for erosion of some coastal areas and accretion in others is illustrated in Fig. 4*A*.

Because of the magnitude of structures required (to be effective in the first instance at the initially large distances offshore), a step procedure seems advisable. For example, a floating offshore breakwater sunk near the coast would cause a tombolo to form, after which a certain quantity of sand would accumulate. The breakwater could then be refloated and moved seawards. This process would need to be continued until the equilibrium shape was obtained as shown in Fig. 4*B*.

With such stable bays no sea defence works would be necessary, because a sedimentary coast can provide a defence which is far superior to any sea walls or revetments devised by man. But to allow for storm-cycles and the short-term reversals of drift, a sufficient width of beach should be allowed as working capital on which the sea can operate. Once the coast has been stabilized, by preventing the net movement of sediment, no long-term erosion need be anticipated and the 'active' beach width can be minimized.

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RADIATION-INDUCED IONIC REACTIONS: THE RETARDATION OF THE HOMOPOLYMERIZATIONS OF α-METHYL STYRENE AND β-PINENE BY WATER

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EVIDENCE has been presented[1,2] that the polymerization of *iso*butene initiated by ionizing radiation is a liquid-phase ionic reaction. It is also stated[1] that high polymer is formed by irradiation of α-methyl styrene at low temperatures, although no experimental results are quoted. As both *iso*butene and α-methyl styrene are examples of monomers that do not readily polymerize by radical mechanisms[3], it is to be expected that their facile homopolymerization would be favoured by an ionic mechanism. Nevertheless some recent communications[3,4] describe the γ-ray polymerization of α-methyl styrene as a radical process, and since the reported inhibitory effects of oxygen and benzoquinone on the radiation-induced polymerization of *iso*butene are equivocal with respect to either radical or ionic mechanisms, we wish to report new evidence in favour of the ionic polymerization of α-methyl styrene and β-pinene carried out *in vacuo* at the ambient temperature, about 30° C., of a cobalt-60 γ-ray source. Both these latter monomers were found to undergo a high degree of conversion to polymer without the marked reduction in rate as a function of amount of conversion that was previously observed for *iso*butene[1]. Again, in contrast to *iso*butene where the isolated polymer had an $\overline{M}_n$ exceeding 100,000 for less than 20 per cent conversion[1], the $\overline{M}_n$ values obtained for α-methyl styrene and β-pinene polymers in this investigation only correspond to about 15 monomer units in each case.

Our earliest experiments on β-pinene[5] established that the polymer yield was sensitive to the pre-treatment of the monomer and particularly to the drying agent employed. Thus it was found that the drying of freshly distilled monomer over sodium, magnesium perchlorate, phosphorus pentoxide and calcium hydride in a large number of separate experiments all gave initial values for G(-monomer) of less than 50, whereas passage of the β-pinene over activated alumina and silica gel gave corresponding polymer yields of 370 and 1,055 respectively. Further, the use of silica gel for drying the liquid within the vacuum apparatus gave the highest yields hitherto recorded for β-pinene, G(-monomer) exceeding 3,000 in some runs. The use of molecular sieve 4*A* (aluminium sodium silicate) as a drying agent for the vapour *in vacuo* gave corresponding G values around 400; but by this procedure the monomer suffered partial isomerization to α-pinene and camphene before irradiation. A confirmation that the polymer yield is affected by the efficacy of the drying agent and not by the removal of other impurities is provided by the effect of deliberately added water to β-pinene; in this instance a sample of water-saturated monomer gave polymer with only a G(-monomer) of 6·8.

A similar conclusion that water exerts a powerful retarding effect on the radiation-induced polymerization of α-methyl styrene is afforded by a comparison of yields obtained with monomer samples which had been first passed down silica gel and then either sealed *in vacuo* or else equilibrated with excess water in a vacuum line prior to seal-off. In the former case the G(-monomer) values measured with a dilatometer were of the order of 10,000 whereas the latter conditions gave an initial G value of about 10. Additional experiments in which concentrations of water below 0·02 weight per cent were added to samples during the course of polymerization showed a marked suppression of the original high rate.

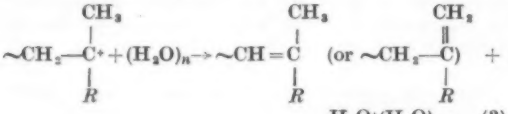
The important role of water in these systems as a polymerization inhibitor cannot readily be explained in terms of the radical reactions (1) and (2):

$$R\cdot + H_2O = RH + OH \qquad (1)$$

$$R\cdot + H_2O = ROH + H \qquad (2)$$

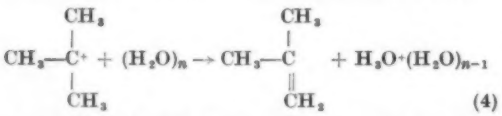
Both these processes are endothermic in the forward direction, and the reverse of (1) is a facile reaction of OH radicals with organic compounds. In any event, these reactions would lead to chain transfer rather than the marked retardation which is observed.

There is no evidence that water acts as a chain transfer agent in radical polymerization in aqueous systems[6]. However, in terms of an ionic polymerization mechanism, it is apt to consider the possibility that reaction (3) represents the interaction of water with a propagating cationic centre.



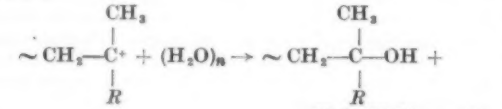
(3)

For the comparable reaction represented by (4):



(4)

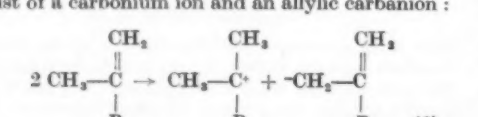
it can be calculated that in the gas phase ($n = 1$) it represents an endothermic process by 27 k.cal./mole. Nevertheless, if the hydronium ion H_3O^+ is fully solvated by surrounding water molecules, reaction (4) becomes exothermic by virtue of the heat of solution of H_3O^+, which amounts[7] to more than 100 k.cal./mole. Franklin[8] has calculated that the forward reaction (4) is 21 k.cal./mole exothermic when all the reacting species are solvated in dilute aqueous solution; by consideration of both heat and entropy changes it was also deduced[8] that the ratio of rate constants for the forward to the reverse reaction (4), at 25° C., is $1{\cdot}1 \times 10^{15}$. Also Evans and Halpern[9] have measured a similar activation energy of 22·6 k.cal./mole for the acid-catalysed addition of methanol to α-methyl styrene which involves a rate-determining step analogous to the reverse of (4) except for the replacement of water by methanol in the reaction. Thus this evidence suggests that the acid-base equilibrium in reactions akin to (3) and (4) in hydroxylic media lies well over to the right-hand side of the equation. Another exothermic process representing the interaction of a carbonium ion with water[9] is illustrated by (5) and represents the familiar fast solvolysis reaction of carbonium ions in aqueous media:



(5)

Similar calculations to those for (4) show[8] that reaction (5) is again highly favoured in the forward direction. We therefore surmise that the theoretical validity of reactions (3) and (5) for the removal of carbonium ions rests on the degree of solvation of the hydronium ion formed.

In a solvent of low dielectric constant it is likely that ions only maintain their existence as ion-pairs. The overall act of radiolysis to form a suitable ion-pair can be represented by (6) where the products consist of a carbonium ion and an allylic carbanion:



(6)

In the case of β-pinene the carbonium and carbanion centres may be located in a rearranged molecule[5]. The path of formation of the ions in (6) could involve the reaction of the parent ion with an olefin molecule to form the carbonium ion and an allylic radical[10,11], followed by electron capture by the latter.

According to this postulate, polymerization proceeds because of stabilized ion-pairs which allow the positive ion a sufficient lifetime to engage in many addition and transfer (to monomer) steps before eventual neutralization by the counter-ion. The presence of water in such a non-polar medium would lead to strong dipole association, whereby dispersed water molecules would be rapidly attracted by electrostatic forces to the vicinity of an ion-pair in a time which has been estimated by Ingold[12] to be less than 10^{-6} sec.; this period is of the same order as the estimated interval between successive propagation steps in the cationic polymerization of *iso*butene[3]. When sufficient water molecules are present in the solvation sheath of the ion-pair, reactions (3) and (5) provide a path for the removal of the positive propagating ion and its replacement by a solvated hydronium ion, thus quenching the polymerization.

In conventional ionic polymerization induced by Friedel-Crafts catalysts, traces of water often function as a necessary co-catalyst to form the initiating complex[13], but there is evidence that further increase in water concentration can lead to a reduction in polymerization-rate[14].

The practical importance of this effect of water in the radiation chemistry of olefins is that latent processes may not be fully realized unless the most rigorous drying of solvents is employed. Divergent yields have been reported by different workers[1,3] for the polymerization of *iso*butene at − 78° C.; since enhanced yields were obtained in this system[15] by the addition of solid powders such as silica gel and zinc oxide, these latter results may well be attributable to the dehydrating effect of such additives. In other work on the homopolymerization of α-methyl styrene, the recorded rates of polymer formation per 10^6 rads have ranged from 0·01 per cent conversion[2] to values between 0·7 and 5·0 per cent[4]. At the same temperature (30° C.) and with comparable dose-rates we have observed considerably greater yields amounting to more than 10 per cent conversion per 10^5 rads; even such yields may in truth be only limiting values, since a finite concentration of water must persist under the driest conditions hitherto employed.

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A PROBABLE FREE-RADICAL MECHANISM FOR SYMBIOTIC NITROGEN FIXATION

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SOME recent very significant experimental data on the evolution and isotopic exchange of hydrogen from nitrogen-fixing root nodules, obtained by Hoch, Schneider and Burris[1], serve to illuminate various possible reaction mechanisms of biological nitrogen fixation. Recent theoretical work[2] on the properties of aqueous nitrogen and current experimental work[3–5] on the physical-chemical properties of certain hæm proteins ('legoglobins'[4], *Lb*, rather than 'leghæmoglobin') associated[6–8] with symbiotic nitrogen fixation, when combined with the information provided by Hoch, Schneider and Burris, has brought to mind a new relatively simple set of hypotheses concerning the first several steps of symbiotic nitrogen fixation. Many of the previous ideas on this subject have been discussed and developed by Roberts[9]. The central hypothesis in the present quite different approach involves formation of the as yet unobserved but thermodynamically predicted[2] free radical N_2H. In conjunction with ideas and facts[3] about the affinity of legoglobins for nitrogen and other simple gases, and knowing[5] that the oxidation potential of iron in ferrolegoglobin is extraordinarily great, the hypothesis of N_2H formation accounts for a diversity of phenomena which is otherwise not understandable at present, as shown below.

Since about 1 per cent of the secondary[3,4] (. . .) hæm sites on hydrated ferrolegoglobin, $Lb\mathrm{Fe}^{+2}\underset{\mathrm{H}}{\mathrm{OH}}\ldots$ (or on oxygenated $Lb\mathrm{Fe}^{+2}\mathrm{O}_2\ldots$), are known[3] to be occupied by nitrogen at 293° K. and 1 atm., it is probable that the first step is :

$$\mathrm{N}_2 + Lb\mathrm{Fe}^{+2}\underset{\mathrm{H}}{\mathrm{OH}}\ldots \rightleftarrows Lb\mathrm{Fe}^{+2}\underset{\mathrm{H}}{\mathrm{OH}}.\mathrm{N}_2 \qquad (1a)$$

or, in aerobic regions of the nodule :

$$\mathrm{N}_2 + Lb\mathrm{Fe}^{+2}\mathrm{O}_2\ldots \rightleftarrows Lb\mathrm{Fe}^{+2}\mathrm{O}_2.\mathrm{N}_2 \qquad (1b)$$

or, on some 'hydroperoxyl sites' produced through the electron affinity of oxygen and subsequent reactions :

$$\mathrm{N}_2 + Lb\mathrm{Fe}^{+2}\mathrm{O}_2\mathrm{H}\ldots \rightleftarrows Lb\mathrm{Fe}^{+2}\mathrm{O}_2\mathrm{H}.\mathrm{N}_2 \qquad (1c)$$

An initial step (1*d*) like (1*c*) except with Fe^{+3} instead of Fe^{+2} is also possible. Because of the noble-gas character of nitrogen, no other possibilities for 'sorption' of nitrogen at room temperature and pressure than reactions of type (1*a*–1*d*) are presently evident ; and it will be shown below that nitrogen must be bound to some other molecule in order that certain fixation reactions can occur. The standard free energy changes for (1*a*–1*d*) are about + 2·4 k.cal./mole [2]. Considering the high concentration of *Lb* in nodules[4,7], this allows a relatively great molar concentration of sites occupied by nitrogen, compared with many enzyme-substrate systems, in spite of the fact that the sites are far from saturated.

A thermodynamically feasible second step is the production of atomic hydrogen, or the somewhat equivalent hydroperoxyl radical water, at the site of nitrogen attachment. This follows from knowing[5] that, at least for one *Lb* species, the oxidation potential for $Lb\mathrm{Fe}^{+2} \rightarrow Ln\mathrm{Fe}^{+3} + e^-$ at *p*H 5·7 is + 30 mV. relative to the standard hydrogen electrode and that this potential has the very large *p*H dependence $\mathrm{d}E^{0\prime}{}_{\mathrm{ox}}/\mathrm{d}(p\mathrm{H}) = +\ 126$ mV./*p*H. That is, in a certain range of low *p*H, ferrolegoglobin itself without any other substrate than the usual hydronium ions in the environment of the protein must be able to liberate appreciable concentrations of hydrogen. For example, at $p\mathrm{H}_{Lb} = 5{\cdot}7$ the free energy change for $Lb\mathrm{Fe}^{+2} + \mathrm{H}^+ \rightarrow Lb\mathrm{Fe}^{+3} + 1/2\mathrm{H}_2$ is − 690 cal./mole when (H^+) and (H_2) are at unit activity ; at $p\mathrm{H}_{Lb} = 5{\cdot}0$ but likewise with $a_{\mathrm{H}^+} = 1$, $a_{\mathrm{H}_2} = 1$, the corresponding free-energy change is − 2720 cal./mole. This means that at equilibrium, $P_{\mathrm{H}_2} = 4{\cdot}4 \times 10^{-7}$ atm. for *p*H = 5·7, and $P_{\mathrm{H}_2} = 1{\cdot}15 \times 10^{-2}$ atm. for *p*H = 5·0, when the activity ratio $\rho = (Lb\mathrm{Fe}^{+3})/(Lb\mathrm{Fe}^{+2})$ is assumed to be $1{\cdot}0 \times 10^{-2}$. If all but 0·5 per cent of the *Lb* is maintained in the reduced state by reactions in the nodule ($\rho = 5 \times 10^{-3}$) then for *p*H = 5·0 the P_{H_2} can reach the relatively high value of $0{\cdot}04_6$ atm., comparable to what was found by Hoch, Schneider and Burris some 300 min. after nodule excision. This range of *p*H values is about one unit lower than the gross average values found[5] in soybean roots and nodules ; but lower values in *Lb*-containing micro-regions of the nodule would be consistent with the strongly acid groups that are associated with the quite low isoelectric point of legoglobins (*p*H 4·4–4·7)[10]. It should be recalled[4,7] that *Lb* exists in rather high concentrations in root nodules.

Not only does the above analysis account for the observed hydrogen production so far as equilibrium may be concerned ; but it also implies the intermediate production of H radicals or their equivalent, in harmony with current ideas about the mechanism of hydrogen liberation from electrodes or polyelectrodes. Thus we expect :

$$Lb\mathrm{Fe}^{+2}\underset{\mathrm{H}}{\mathrm{OH}}\ldots + \mathrm{H}^+_{\mathrm{aq.}} \rightleftarrows Lb\mathrm{Fe}^{+3}\underset{\mathrm{H}}{\mathrm{OH}}\ldots + \mathrm{H} \qquad (2a)$$

or, in aerobic regions :

$$Lb\mathrm{Fe}^{+2}\mathrm{O}_2\ldots + \mathrm{H}^+_{\mathrm{aq.}} \rightleftarrows Lb\mathrm{Fe}^{+2}\mathrm{O}_2\mathrm{H}\ldots \rightleftarrows Lb\mathrm{Fe}^{+2}\underset{\mathrm{H}}{\mathrm{OH}}\ldots + \mathrm{HO}_2 \qquad (2b)$$

Note that hydrogen atoms would be produced at the same site that promotes nitrogen attachment, a situation very favourable kinetically for a reaction between nitrogen and hydrogen atoms, even though at equilibrium the hydrogen atom concentration must be very small relative to hydrogen. Furthermore, even in the presence of some oxygen a high reductive potential can be maintained by *Lb*, accord-

ing to the results of George and Stratmann[11] for the quite similar myoglobin.

The third step would then be a direct or indirect combination of nitrogen and hydrogen atom, the simplest being :

$$Lb.N_2 + H \rightarrow Lb.N_2H \qquad (3)$$

In equation (3) $Lb.N_2$ represents any one of the three complexes produced in (1*a*), (1*b*) or (1c). The reason for emphasizing the existence of a complex in equation (3) is to bring out the fact that a free radical and a molecule may not combine without some means of dissipating the binding energy ; in this case the known forces[3] exerted by the hæm protein on nitrogen ('secondary gasation') can serve such an essential purpose. It has already been shown[2] that the standard reaction for $N_2 + H_2 \rightarrow N_2H$ would be spontaneous ; and that a standard free energy investment of 32 k.cal./mole would be required to break the $N_2..H$ bond. Furthermore, as reaction (3) proceeds it would have to shift equilibrium (2*a*) and lead to the appearance of (oxidized) ferrilegoglobin, which is what Hamilton, Shug and Wilson[12] found in reduced nodule extracts when helium was replaced by nitrogen. However, equilibrium (2*a*) must be maintained with a preponderance of *Lb* in the ferrous form for an appreciable yield of hydrogen. This is in harmony with the observations of Keilin and Smith[7] that at all stages of nodule development no ferrihæm proteins were observed ($\rho \gtrsim 10^{-2}$). In order to maintain such a favourable reduction potential at the iron site, some coupling of legoglobin with an electron transport system is required ; and such a coupling is likely according to Falk, Appleby and Porra[13]. Also, an essential link in such an electron transport system having a one-electron transfer at one end is very probably a molybdoflavoprotein[14], especially since molybdenum is a specific requirement for nitrogen fixation. Nevertheless, a molybdenum reduction site would not be adequate for nitrogen fixation even though its reduction potential for hydrogen atom formation were more favourable than that of an $Lb\text{Fe}^{+2}$ site, because the concentration of molybdenum in the nodule is too small to furnish an adequate number of sorption sites for the weakly bound nitrogen.

Subsequent steps for the successive addition of hydrogen atoms to N_2H_n are obvious and thermodynamically feasible[2], leading to the eventual production of hydrazine, in harmony with the elegant nitrogen-15 tracer experiments of Bach[15]. However, alternative pathways including even oxidative steps[2] may develop at this or even earlier stages owing in part to the possibility[2] of a direct succession of electron, proton captures by N_2H_n. Of course, once N_2H_4 has been formed, the subsequent reduction to $2NH_3$ follows easily through conventional reactions or perhaps through a hydrazine reductase, such as a molybdoflavoprotein. The present view is thus consistent with the idea stressed by P. W. Wilson and R. H. Burris[14] that NH_3 is the "key intermediate" between inorganic and organic nitrogen.

Innumerable side or parallel reactions will undoubtedly develop as soon as N_2H is produced, especially under aerobic conditions. Only two of these need be considered here, to illuminate the possible need[2] for a reinterpretation of the usual Michaelis–Menten constant of the symbiotic nitrogen-fixing process. The back-reactions :

$$2N_2H \rightarrow 2N_2 + H_2 \qquad (4a)$$

or

$$2N_2H \rightarrow N_2 + N_2H_2 \qquad (4b)$$

are thermodynamically feasible[2], and imply rate laws second order with respect to N_2H, meaning that as N_2H production increased, that is, as occupation of active sites by nitrogen increased, a point would be reached where the back reactions would cut down drastically on the increase in net fixation-rate with increasing P_{N_2}, otherwise expected when the reactive sites are not saturated. Thus the apparent fact that the "N_2-fixing complex in nodules is half-saturated at a P_{N_2} of about 0·025 atm.[1]" may actually reflect, in part, the effect of back reactions (4*a*) and (4*b*) going on at a considerable rate. This consideration helps one to understand how the N_2-fixation rate is not appreciably enhanced above $P_{N_2} \approx 0{\cdot}05$ atm., even though only about 1 per cent of the total hæm sites of all kinds are occupied. Further consideration shows, however, that of these the hydroperoxyl site, probably the most active type, must be almost fully saturated at $P_{N_2} = 1$ atm. if the effect of hydrogen, of nitrogen and of the binding of $O_2..H..N_2$ are taken into account.

The above hypotheses explain a number of striking facts about symbiotic nitrogen fixation, some of which are discussed briefly below :

(1) *N_2-nitrogen inhibits hydrogen evolution*[1]. The formation of N_2H would divert hydrogen atoms from their normal fate of eventually combining, in association with some third body, for example, protein, to form hydrogen molecules.

(2) *N_2-nitrogen is essential for promoting an appreciable exchange reaction between deuterium and endogenous hydrogen*[1]. The presence of free N_2H would allow the exchange $N_2H + D_2 \rightarrow N_2HD + D$, among others, because the relatively weak binding free energy between nitrogen and hydrogen atoms (32 k.cal./mole) implies that the H in N_2H is relatively free to act as a kind of quasi H-atom ; and $H + D_2 \rightarrow HD + D$ is known[16] to occur readily even in the low-temperature range of + 10° to 100° C.

(3) *H_2-hydrogen inhibits the evolution of hydrogen, itself*. From the present point of view, the evolution of hydrogen would probably depend on two slow processes in series : (*A*) Production of hydrogen atoms from $Lb\text{Fe}^{+2}$; and (*B*) conversion of the hydrogen atom to the molecule, either by (*a*) a very slow process of two hydrogen atom migrations or attachments to the same third-body site, analogous to the probable mechanism giving rise to hydrogen over-voltages on electrodes, and/or (*b*) a more rapid process near the reactive site such as $Lb\text{—}H_n + H \rightarrow Lb\text{—}H_{n-1} + H_2$, a type common to free radical reactions. Recent preliminary work in this laboratory[5] indicates that nodules treated with hydrogen slowly yield a different, more stable, type of ferrolegoglobin $(Lb\text{Fe}^{+2})_{H_2}$ than that extracted under argon $(Lb\text{Fe}^{+2})_A$ or under air $(Lb\text{Fe}^{+2})_a$. The $(Lb\text{Fe}^{+2})H_2$ had an oxidation potential smaller than that of $(Lb\text{Fe}^{+2})_a$ by about 40 MV. ; and at 30° C. the $(Lb\text{Fe}^{+2}O_2)_{H_2}$ auto-oxidized to $(Lb\text{Fe}^{+3})_{H_2}$ only about one-half as fast as fresh $(Lb\text{Fe}^{+2}O_2)_A$. Also, in the potentiometric titration of $Lb\text{Fe}^{+2}$ with phthiocol, several equivalents of hæm-protein side-groups, as well as iron, were easily reducible. Hence I tentatively conclude that hydrogen molecules will convert $Lb\text{Fe}^{+2}$ to a less-reactive form which will at

least slow down step (*A*) above; and possibly alter the rate of step (*B*) as well. This would mean that the intrinsic evolution of hydrogen, itself a possibly unavoidable by-product of the step that produces the essential hydrogen atoms, may eventually lead to a substantial reduction of nodule effectiveness.

(4) *H_2-hydrogen is a specific competitive inhibitor of N_2-fixation.* (*A*) Hydrogen is known[3] to attach itself to legoglobin, with a standard uptake free energy of about + 2·3 k.cal./mole, in a way quite similar to that of nitrogen. If hydroperoxyl sites were the most active type, even though the least abundant, the fixation-rate in the absence of hydrogen would be characteristic of secondary sites nearly saturated with nitrogen because of specific $O_2..H..N_2$ binding forces. In this case the rate would be decreased through displacement of nitrogen by hydrogen. (*B*) The conversion of *Lb*Fe^{+2} to a hydrogenated form that produces atomic hydrogen at a lower rate, evidence for which is given in (3), must also result in a decrease in N_2H formation, that is, in rate of nitrogen fixation. (*C*) To some extent molecular hydrogen would also complete for the bound HO_2 radicals, analogous to the competition for OH radicals postulated by Pethica, Roberts and Winter[17], but this must be a second-order effect in view of the slow molecular deuterium exchange-rate in the absence of nitrogen.

(5) *Carbon monoxide inhibits nitrogen fixation but is not primarily a competitive inhibitor.* (*A*) The known attachment of carbon monoxide to legoglobin, forming *Lb*$Fe^{+2}CO$..., would not destroy the secondary sites for nitrogen attachment[3,4] except at extreme carbon monoxide pressures, though it would alter (probably lower) the oxidation potential of *Lb*Fe^{+2}; (*B*) The principal effect of carbon monoxide should be to divert hydrogen atoms away from N_2H formation, according to the known[16] complicated reactions: $H + CO + M \rightarrow HCO$, followed by a set of reactions producing formaldehyde, glyoxal and polymers; and possibly by another parallel set regenerating atomic and molecular hydrogen.

(6) *Carbon monoxide strongly inhibits exchange of endogenous hydrogen with deuterium.* The reactions in (5) (*B*) incorporate hydrogen atoms into compounds much more stable than N_2H, hence are much less likely to furnish exchangeable quasi hydrogen atoms. This implies that those reactions which might regenerate atomic and molecular hydrogen from HCO in the gaseous state are not appreciable under conditions prevailing in the nodule.

(7) *O_2-oxygen is a requirement for symbiotic nitrogen fixation, but there is some inhibition by oxygen at high pressures.* Hydroperoxyl sites on ferrolegoglobin (equation (1c)) are probably the most active though the least abundant type; and also should be nearly saturated by nitrogen at 0·1 atm. This follows from the presence of a loosely bound atomic hydrogen on the hydroperoxyl site adjacent to the secondary site; and from the known affinity between atomic hydrogen and nitrogen. Also, the standard free energy for the change $N_2 + HO_2 \rightarrow N_2H + O_2$ is probably[2] of the order of only a few k.cal./mole but negative. At high values of P_{O_2} the secondary sites could just as well be occupied by oxygen as by nitrogen (that is, by molecules of similar size, shape and polarizability), according to the theory of secondary gasation[3,4]. Of course, the above considerations are not complete or necessarily definitive because of the influence of oxygen on bacterial metabolism.

It must be emphasized that some of the properties of legoglobin referred to in the present discussion may not represent the exact behaviour of the native material within the intact nodule. This is underlined by the fact that legoglobin oxidizes[7] and autooxidizes[5] rapidly when extracted aerobically, that it is susceptible to change in the presence of hydrogen and that it is sensitive to dialysis and changes of *p*H and ionic strength[5]. There can be little doubt that the properties of the native legoglobins must be known before the chemical processes in nitrogen-fixing nodules are understood, in accordance with Keilin's early ideas are in harmony with the recent work of Falk, Appleby and Porra[13] showing a correlation between deficiencies of hæm synthesis within the nodule and ineffectiveness of nodules in respect to nitrogen fixation.

It may be concluded that the present set of hypotheses provide a consistent way of viewing most of the central qualitative and semiquantitative aspects of symbiotic nitrogen fixation, excluding for the time being the more difficult questions about the magnitudes of the various rate constants. In view of the complexity of interrelated facts that must be explained, it is difficult at this stage to see an alternative set of hypotheses that could differ radically from the present ones. It would appear worth while to look for the free radical intermediates suggested here, using the powerful high-resolution electron spin resonance techniques now available in some laboratories; but keeping in mind that the free radical spectrum of the expected water, atomic hydrogen, N_2H_n and *Lb* intermediates as a function of time will undoubtedly be most complex. The liberation of molecular hydrogen from aqueous legoglobin and the detection of hydrogen atom radicals in that process would also be an important test of the present hypotheses. Eventually, of course, it should be possible to find conditions for the fixation of nitrogen through the mediation of a protein molecule having properties at least very similar to those of the legoglobins.

I am grateful for support from the Herman Frasch Foundation and the Utah Agricultural Experiment Station in this work.

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INTERACTION ('PERIPOLESIS') OF MACROPHAGES AND LYMPHOCYTES AFTER SKIN HOMOGRAFTING OR CHALLENGE WITH SOLUBLE ANTIGENS

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IN conjunction with an investigation of the lymphoid reactivity to tissue homografts[1], we have recently examined the behaviour of living cells in cultures of lymphoid tissue taken from rabbits which either had received skin homografts or had been challenged with an intravenous soluble antigen.

The explants of lymphoid tissue were set up in a simple culture chamber[2] in plasma clot, with a fluid medium composed of equal parts of human serum and a chemically defined medium[3]. The activity of the cells, and especially of the macrophages and lymphocytes, in the outgrowth from these explants was recorded on 16-mm. film by time-lapse phase-contrast cinemicrography, at 12 pictures per min.

In the initial experiments homografts of skin 2 cm. in diameter were applied to the ventral aspect of the ears, 1–2 cm. from the external auditory meatus, in thirteen young adult male rabbits. Eight to ten days later the first regional (parotid) lymph node draining the area of the graft was removed aseptically. Since it was known that the upper half of the node was more likely to have received lymph from the grafted area and to contain the reactive cortex[1], tissue from this region was taken for culturing. A total of about 120 explants from these lymph nodes were cultured. The effect of a soluble antigen has also been examined by challenging seven rabbits with a single intravenous injection of 5 ml. of horse serum. Three to twelve days later the spleens were removed for culture; a total of about 150 explants were obtained from the seven animals. Control cultures were provided by about 100 explants of spleen from five normal rabbits which had not been exposed to the antigen.

When the cine film of the cultures of the antigenically challenged lymph nodes and spleens was studied, an association was noticed between some of the macrophages and small lymphocytes. The association consisted of the movement of lymphocytes close to the surface of particular macrophages. The cells concerned showed their normal motility during the association, the macrophages moving slowly and at random, while the contiguous lymphocytes continued to move relatively rapidly around the edge and over the upper free surface of the macrophage. This association was seen to persist for prolonged periods; during most of this time the contact between lymphocytes and macrophages appeared to be of a surface-to-surface nature, although the more rapidly moving lymphocytes would at times move away, only to return again to the macrophage.

Prolonged contacts of this nature were not seen in the control cultures of spleen. Though random contacts between macrophages and lymphocytes occurred quite frequently, such contacts never persisted for more than a few minutes.

Figs. 1–4 are enlargements from the original 16-mm. film. In Fig. 1 the arrow indicates a small lymphocyte in close association with the macrophage immediately above, in a culture of lymph node which had drained a homograft of skin. Fig. 2 shows the same two cells, still in contiguity, about 5 hr. later. Figs. 3 and 4 illustrate a similar association between a group of lymphocytes and a macrophage in an explant of spleen which had been challenged intravenously with horse serum. The lymphocytes are so closely packed that they largely obscure the macrophage which is only partially visible. The time-interval between Figs. 3 and 4 is approximately 75 min.

An association between normal small lymphocytes and cells in mitosis, megakaryocytes and malignant cells, has been recorded by Pulvertaft and his colleagues[4,5]. They examined explants of normal and pathological human lymph nodes and bone marrow *in vitro* using time-lapse phase-contrast cinemicro-

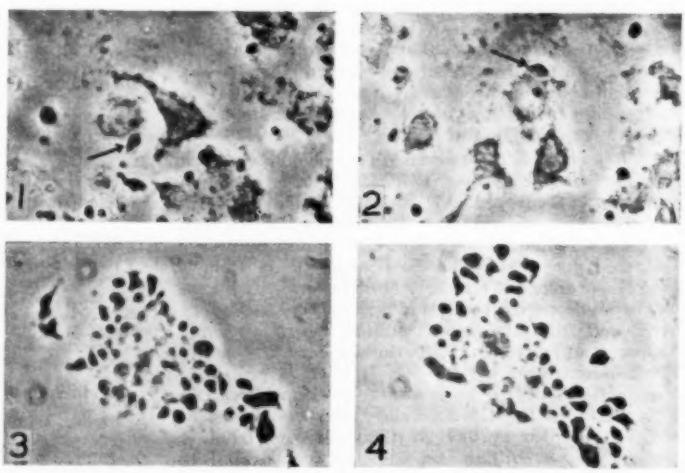

Fig. 1. (1) Cells in the outgrowth from an explant of lymph node which had drained a homograft of skin. Two days *in vitro*. (2) The same cells 5 hr. later. (3) A group of lymphocytes surrounding and partially obscuring a macrophage. Explant of spleen from rabbit injected intravenously with 5 ml. horse serum. Two days *in vitro*. (4) The same field 75 min. later

graphy, and, in many instances, observed the entry of normal small lymphocytes into the cytoplasm of living malignant cells. The cytoplasmic invasion of epithelia, fibroblasts, endothelial cells and ova by small lymphocytes is now firmly established[6]. For these phenomena wherein lymphocytes become adherent to other cells or apparently pass over or into them, Pulvertaft and his colleagues have suggested the name 'emperipolesis' from the Greek 'εμπεριπολησις'—'inside round about wandering'.

The interaction between normal small lymphocytes and macrophages has not been recorded previously, but an association between 'malignant' lymphocytes and macrophages has been shown to occur in tissue obtained from humans with reticuloses[5] and from mice with certain lymphocytic tumours[7].

In our experiments there was no evidence of the entry of small lymphocytes into the cytoplasm of the macrophages. We suggest, therefore, that to this phenomenon of free lymphocytic movement around the perimeter of a cell for hours at a time but without entry into its cytoplasm, the name 'peripolesis' be applied.

The significance of peripolesis is by no means clear. The pattern of our experiments and the results observed would suggest that the phenomenon might be related to the early (radio-sensitive) phase of antibody production. It seems improbable that the foreign human proteins present in the culture medium could be involved in the cellular interaction, as peripolesis was not observed in the control cultures. It might be that the lymphocytes are transferring nutritive substances[8] necessary for antibody production to the macrophages. An antigen-antibody attraction, which might be the explanation of the association which Nossal[9] observed between *Salmonella adelaide* and sensitized plasma cells, cannot be excluded as an explanation of peripolesis. The final possibility exists that the macrophages, having taken up antigen from the extra-cellular fluid *in vivo*[10], are in the process of supplying an antigen-complex to the lymphocytes which then stimulates antibody production.

Though it is now established that the plasma cell series synthesizes antibodies[10,11], other workers have suggested that the stem cell of antibody production may be the macrophage[10,13], primitive reticular (mesenchymal) cell[12] or lymphocyte[12]. Our observations are consistent with those of Roberts *et al.*[12], who showed that subcutaneous or intramuscular injections of cell suspensions containing both macrophages and lymphocytes into X-irradiated immunologically inert rabbits would result in the transformation of these cells, after antigenic challenge, into large pyroninophilic antibody-producing cells.

The suggestion[10] that the macrophages may provide the first link in a chain leading to antibody production would explain the prolongation of skin homograft survival after injection of trypan blue[14], by temporarily impairing the capacity of macrophages to take up antigen derived from the graft.

The phenomenon of peripolesis in antigenically challenged animals is being investigated at the present time and will form the basis of a communication in the near future.

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VISUAL PIGMENT FROM A PURE-CONE RETINA

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ONLY one of the numerous visual pigments that have been extracted to date from the retinæ of vertebrates has any claim to be regarded as a cone pigment[1]. This is iodopsin[2], discovered by Wald in 1937 in the chicken retina. Extracts of chicken retinæ contain a mixture of photosensitive pigments: iodopsin with $\lambda_{max.} = 562$ mμ and a rhodopsin with $\lambda_{max.} =$ about 500 mμ. Since the rhodopsins are known to occur in some pure-rod retinæ, and since the chicken retina is rich in cones as well as rods, it has been assumed that the chicken pigments are not mixed *in vivo* but exist separately, the rhodopsin being in the rods and the iodopsin in the cones.

According to the Duplicity Theory, scotopic vision is mediated by rods, and photopic vision by cones. Consequently, the fact that the scotopic and photopic visual functions of the chicken, as measured by Honigmann[3], roughly correspond to the absorption spectra of chicken rhodopsin and iodopsin also supports the view that these pigments occur in the rod and cones respectively.

These arguments for believing iodopsin to be a cone pigment, though practically compelling, are nevertheless indirect. Until it becomes possible to divide rods from cones and to extract them separately, some doubt remains. Such doubt, which may seem trifling in the case of iodopsin, can be a serious obstacle to interpretation in other instances[4] where extraction of a mixed rod-cone retina likewise yields a mixture of photosensitive pigments but where (unlike the chicken) no sensitivity functions have been measured to provide a guide in allocating the pigments.

In some species of nocturnal animals, the retinæ are of the pure-rod type. In such cases any extractable visual pigment can be confidently assumed to be a rod pigment. Even when a few cones are also present in an otherwise pure-rod retina (for example, rat), this is still held to be a safe assumption, for it is commonly

supposed that the amount of cone pigment is small in comparison with the amount of rod pigment.

Conversely, in those diurnal species which have pure-cone retinæ one could assume that any extracted visual pigment is a cone pigment. Unfortunately, there are not many species which possess such retinæ, and all attempts to obtain visual pigment from them have so far proved unsuccessful[1]. Among the well-authenticated cases of a pure-cone retina is that of the grey squirrel (*Sciurus carolinensis leucotis*)[5]. This article briefly describes a recent successful attempt to extract visual pigment from it.

Extraction of the Squirrel Retinal Pigment

In August 1959, a batch of 40 grey squirrels was received. Between that date and February 1960, several retinal extracts were prepared, the procedures used being varied slightly in order to determine the best conditions for obtaining the visual pigment. The squirrels were fed throughout on standard 41*B* diet with occasional carrot and cabbage. For the first six weeks they also had mixed cereal. The animals thrived on this diet, and the last representatives of the batch weighed about twice as much as when they were first received.

The best extraction procedure found so far is given in the following account of one of the final experiments. Nine squirrels, of total weight 5·9 kgm., were dark-adapted overnight (17 hr.). All subsequent operations were also conducted in the dark room with the aid of a deep red safe-light. The squirrels were first anæsthetized by chloroform. Each animal was then killed by an intraperitoneal injection of nembutal. The eyes were removed and washed in water. Peripheral incisions at about the level of the ora serrata were next made in each eye. The front of the eye, including the lens, could then be lifted away leaving the posterior hemisphere (which contained the retina) full of vitreous humour. The next step was to slide forceps under the retina (which was quite loose) and to grasp it firmly at its junction with the optic nerve. The retina, lying across the limbs of the forceps, was then gently pulled away whole with its vitreous, and placed in McIlvaine's *p*H 7 buffer solution. With gentle shaking, the rather adherent vitreous separated from the retinæ, so that by subsequent centrifuging they were precipitated, leaving blobs of vitreous floating in the supernatant buffer solution. This was removed. Sucrose solution (40 per cent) was then added to the retinæ, and the mixture vigorously shaken. This caused the outer segments of the visual cells (cones) to break away from the retinæ. On centrifuging the mixture, the heavier retinal debris was precipitated to the bottom of the tubes, leaving a slightly cloudy supernatant suspension of visual cells in the sucrose solution[6]. This was pipetted off. A further yield was obtained by shaking the retinal residues a second time with sucrose solution, and repeating the procedure.

The visual cells were precipitated by diluting the suspensions with three volumes of McIlvaine's *p*H 4·6 buffer, and centrifuging them for 35 min. at 4,000 r.p.m. The clear supernatants were then pipetted off, leaving behind compact sediments of visual cells. These were bulked into a single tube with the aid of 4 ml. of *p*H 4·6 buffer (removed after further centrifuging). The combined outer limbs were then extracted with 0·5 ml. of 3 per cent digitonin for 1 hr., after which time the mixture was centrifuged and the clear extract withdrawn and made alkaline by the addition of 0·05 ml. of saturated sodium borate solution (first extract). Further extraction with another 0·5 ml. of the digitonin solution, this time for 25 hr., yielded a second extract which was made alkaline as before. The two extracts were a pale pink colour and faded to pale yellow on exposure to light. The spent cells were pure white. In order to obtain information on the total amount of visual pigment in the squirrel retina (see below), the retinal residues were likewise extracted twice with 0·5 ml. portions of 3 per cent digitonin. These extracts were naturally more contaminated and contained only a trace of visual pigment.

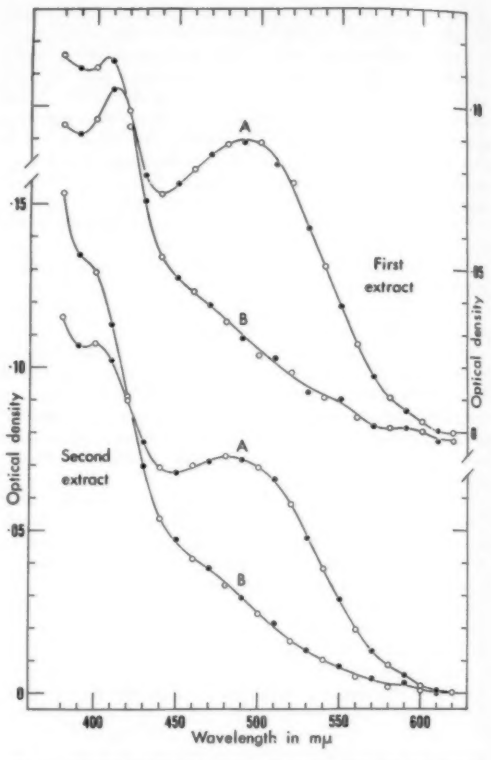

Fig. 1. Density spectra of the first and second extracts of squirrel visual cells before (*A*) and after (*B*) total bleaching. The outward measurements from 380 mμ to 620 mμ are given by the empty circles; the return intervening measurements, by the black circles. Measurements were made at 35·5° C. on the first extract (at lower temperatures the extract became reversibly cloudy), and at 20° C. on the second extract

Nature of the Squirrel Retinal Pigment

The density spectra of the first and second extracts before and after complete bleaching by a 15-W. lamp are given by the curves in Fig. 1. From the shapes of these curves it is clear that, in spite of the washing procedures described above, blood pigment and other impurities were present, in addition to visual pigment. Because of this contamination (relatively less in the first than in the second extract) the maxima for the unbleached extracts—at 490 and 480 mμ respectively—lie at shorter wave-lengths than would otherwise have been the case.

By presenting the results in the form of a difference spectrum (curve for unbleached, minus curve for bleached extract) the contribution of stable impurities

is eliminated. Moreover, a difference spectrum for an alkaline extract normally has its maximum at a wave-length only slightly longer than that for the pure visual pigment. The difference spectrum for the squirrel pigment (second extract) is shown by the circles in Fig. 2. It has $\lambda_{max.} = 504$ mμ, and agrees closely with that found by Bridges[7] under similar conditions for the hamster pigment (crosses). This latter is a 'rhodopsin', that is, a visual pigment based on retinene$_1$. Bridges gives the maximum for the pure hamster pigment at 502 mμ, and we may conclude that the squirrel pigment must be very similar.

None of the extracts prepared in the present investigation was sufficiently dense to allow a useful analysis by the method of partial bleaching[8]; consequently, the homogeneity of the squirrel pigment could not be definitely established. All extracts, however, yielded difference spectra identical within experimental error with that shown in Fig. 2.

The fully bleached extracts showed an unexpectedly high absorption in the 450–550 mμ range (see Fig. 1). This high absorption cannot be ascribed to the Soret band, nor to unbleached visual pigment (since the extracts were exposed to light until no further changes took place). Consequently it must be due to a photo-product. Some information on this may be obtained as follows. At wave-lengths longer than 500 mμ, the contribution of impurities to the density spectrum of an unbleached extract is small. Consequently, with the aid of the visual pigment nomogram[9] it is possible, from this spectral region, to obtain an estimate of the amount of pure visual pigment present. By subtracting the density spectrum appropriate to this amount from the 'unbleached' curve, a remainder is left which is the contribution of the impurities. If this same contribution is now subtracted from the curve for the bleached extract, we are left with the density spectrum for the pure photoproduct. When this is done with the curves of Fig. 1, it is found that, in addition to a rising extinction towards the short-wave end of the spectrum, the pure photoproduct has a low maximum at about 480 mμ. Thus the photoproduct of the squirrel pigment includes a 480-mμ pigment as well as the normal retinene$_1$, or retinene$_1$-protein, typical of rhodopsins in solution.

The presence of a 480-mμ pigment in the products of bleaching would tend to displace the difference spectrum towards longer wave-lengths. The amount of 480-mμ pigment was small, however, and the displacement caused must be correspondingly slight. At the least we may say that the true maximum of the squirrel pigment must lie between 490 mμ, the $\lambda_{max.}$ of the least-contaminated extract, and 504 mμ, the $\lambda_{max.}$ of the difference spectrum.

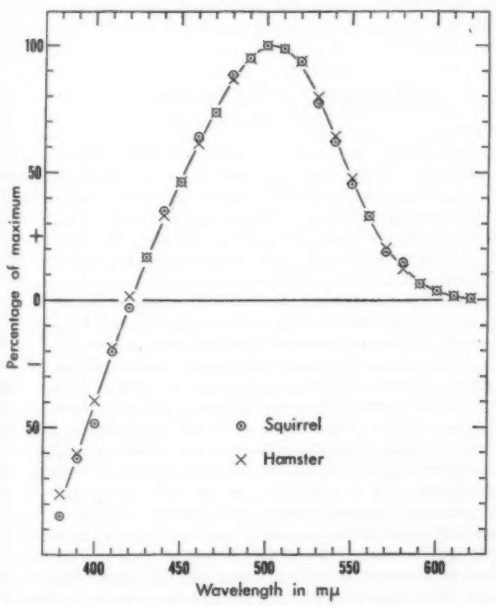

Fig. 2. Comparison between the difference spectra (alkaline conditions) of the squirrel and hamster visual pigments. Squirrel data (20° C.) from second extract; hamster data (25° C.) from Bridges (ref. 7)

Interpretation of *in vivo* Observations on the Squirrel Retinal Pigment

The retinal pigment of the grey squirrel has been studied by Weale[10] using the method of fundus reflectometry. He measured the fundus reflexion spectrum before and after exposure of the living retina to bright white light. The changes caused by the exposure were interpreted as due to the bleaching of a visual pigment, characterized by a very sharp absorption band with maximum extinction at 530–540 mμ.

How can this result be related to the present finding that extraction of the visual cells of the squirrel retina with digitonin solution yields a typical 'broad-band rhodopsin' with maximum extinction at about 500 mμ?

There are no grounds for supposing that the discrepancy could be due to a gross difference between the light-absorbing properties of the pigment in the *in vivo* and extracted conditions. I have shown that the rhodopsin of the frog (visual pigment 502) has precisely the same absorption in the visual cell as it has after extraction into digitonin solution[1]. This is true also of two other pigments which have been compared in this way, namely, the retinene$_1$ pigment of the conger eel (visual pigment 487) and the retinene$_2$ pigment of the carp (visual pigment 523) (Dartnall, unpublished work).

In any event, Weale (as he was aware) measured the difference spectrum of the squirrel pigment, not its direct absorption. Difference spectra (depending on the conditions under which they are obtained) may, or may not, be useful guides to the absorption spectra of the original visual pigments. In alkaline solution, for example, there is generally little overlap, in the visible, between the absorptions of visual pigments and their photoproducts. The positive portions of difference spectra consequently give good approximations to the original pigment absorptions. When the visual pigment is *in situ*, however, this is not the case. Thus a visual cell preparation of the frog pigment 502 can yield a difference spectrum maximal at 510–515 mμ (ref. 11). This is because a substantial amount of a stable 470-mμ pigment is among the photo-products formed under these conditions[12]. Similarly, visual cell preparations of the conger pigment 487 and the carp pigment 523 likewise yield 'displaced' difference spectra, with maxima at 502 and 535 mμ respectively (Dartnall, unpublished work).

Reference has been made above to the presence, in bleached squirrel extracts, of a small amount of 480-mμ pigment (in addition to the 'normal' retinene$_1$-protein). It is considered likely that in the water-free

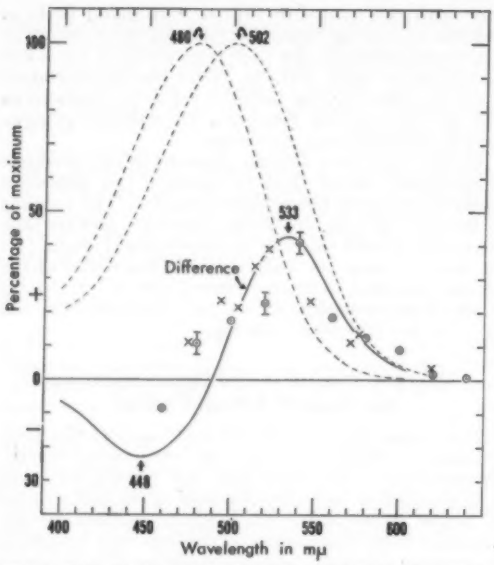

Fig. 3. Interpretation of *in vivo* measurements of the squirrel pigment and of the spectral sensitivity function. Circles (with bars indicating standard deviations) are Weale's results (ref. 10) for the density changes on bleaching the squirrel retina; crosses are Arden and Tansley's mean measurements of spectral sensitivity (equal quantum—intensity spectrum, corrected for lens absorption) (ref. 5). Both sets of results are scaled so that the maxima agree with that of the difference curve, that is, the difference between the 502 and 480 curves

environment of the visual cell this substance may be formed in proportionately greater amount than in solution. If so, then a difference spectrum obtained *in vivo* would bear little resemblance to the spectrum of the original pigment.

In Fig. 3 an attempt has been made to formulate this idea in a semi-quantitative manner. The curves marked '502' and '480' represent the absorption spectra of the squirrel pigment and its 480-mμ photoproduct respectively. The curve marked 'difference' was obtained by subtracting the '480' curve from the '502' curve. It has a positive maximum at 533 mμ and a negative maximum at 448 mμ. Weale's results[10] (shown by the circles in Fig. 3), when suitably scaled, agree tolerably well with this difference curve.

The shape (as distinct from the magnitude) of the difference curve is not markedly affected by the precise spectral location of the pigment and photoproduct curves. (Thus were we to assume a photoproduct at 470 mμ instead of at 480 mμ, the positive and negative maxima of the difference curve would be at 527 and 446 mμ respectively: for a 490-mμ photoproduct they would be at 537 and 452 mμ respectively.) Nor is its shape much affected by quite considerable variations in the relative heights of the pigment and photoproduct curves (arbitrarily shown equal in Fig. 3). We may conclude that the difference spectrum obtained by Weale in his *in vivo* experiments is consistent with that to be expected when a normal broad-band visual pigment having maximum extinction at about 500 mμ bleaches mainly or entirely to a substance having maximum extinction at about 480 mμ.

Amount of Pigment in the Squirrel Retina

At the wave-length of maximum change (*c.* 535 mμ), Weale observed a difference of density of 0·16 between the unbleached and bleached retina. Assuming that the light by which this difference had been measured had passed twice through the pigment layer, he originally[10] supposed the pigment density to be half this figure. One cannot be certain, however, that the measuring light does make such a double transit[12]. Consequently, it is safer to regard the values 0·08 and 0·16 as lower and upper limits respectively.

If the interpretation proposed in Fig. 3 for Weale's results is correct, it is clear that his maximum change amounts only to some 40 per cent of the density of the squirrel pigment. Allowance for this factor raises Weale's estimate from 0·08–0·16 to 0·20–0·40.

It is interesting to compare this value (a measure of the effective absorptivity of the pigment when in the retina) with that for the pigment when extracted. In the most efficient of the present experiments, nine squirrels were used, the isolated visual cells being twice extracted with digitonin solution (first and second extracts of Fig. 1). The residual retinæ were likewise extracted twice. Adding the density results for all four extracts gives the total pigment yield as 0·55 ml. of extract of density 0·182 (per 0·5 cm.). Assuming the retinæ to be hemispheres of 1·15 cm. diameter, it is calculated that such a quantity of pigment would, if spread uniformly over the whole retinal area, cover it to an optical density of 0·0054.

The two values disagree grossly. Even after discounting a possible 3/2 factor[14] for increased absorptive efficiency due to orientation of the pigment molecules in the cones, the *in situ* estimate is still 25–50 times as great as that from extraction results.

Estimates of *rod* pigments *in situ* and after extraction normally agree fairly well when allowance for orientation and for the fraction of retinal area occupied by the rods[14] is made. The present discrepancy with the squirrel *cone* pigment shows that the effective absorptivity of the pigment is greatly enhanced *in situ*, for example, by a mechanism which concentrates all the light incident on the inner segment of a cone into the smaller, pigment-containing area of the outer segment[15].

Interpretation of the Squirrel Spectral Sensitivity Curve

Arden and Tansley[5] have determined the spectral sensitivity of the grey squirrel from measurements of electroretinograms taken from animals under light nembutal anæsthesia. Their mean values (from two dark- and two light-adapted squirrels) are shown by the crosses in Fig. 3, and are seen to be in tolerable agreement with the difference curve.

This agreement suggests that the spectral sensitivity function may be mediated by a mechanism depending on the difference between the light absorbed by visual pigment chromophores and that absorbed by '480-' chromophores. The precise way in which this could occur is a matter for conjecture at present. One possibility, which has the merit of suggesting crucial electroretinogram experiments, is outlined below.

Hubbard and Kropf[16] have suggested that when rhodopsin (in a dry film or at low temperature) is irradiated, "steady state mixtures of chromoproteins including photoregenerated rhodopsin and iso-rhodopsin" are formed. We shall postulate that in the water-free environment of the cone a similar situation arises, and that continuous irradiation results in a steady-state mixture of the 480-mμ pigment and photoregenerated squirrel rhodopsin. In other words, we postulate that the 480-mμ pigment formed when

squirrel rhodopsin bleaches is similarly photosensitive in the cone, and reverts totally or partially to the original pigment on absorption of light quanta.

Consider a cone which (as a consequence of its past history of exposure to light) contains approximately equal densities of 480- and 502-mμ pigments. If, now, this cone is exposed to light of wave-length about 490 mμ (which both pigments absorb equally, see Fig. 3) then the net changes in the pigment composition of the cone will be nil, for, on average, just as many 502-chromophores will be lost through bleaching as will be gained by photo-regeneration. Exposure of such a cone to any wave-length longer than 490 mμ will result in a net increase in 480-chromophores. Conversely, exposure to any wave-length shorter than 490 mμ will cause a net increase in 502-chromophores.

If we suppose that it is the change in chromophore constitution of the cone which ultimately provides the stimulus, then the sensitivity would be given by the *modulus* of the difference curve in Fig. 3. This modulus is a double-humped curve with maxima at 448 and 533 mμ and with a deep depression between, touching zero at about 490 mμ. Unfortunately, Arden and Tansley's observations were not extended far enough into the short wave-lengths to ascertain whether there is a second maximum of activity at about 450 mμ.

It has already been noted that the shape of the difference curve is not markedly affected by quite large variations in the relative heights of the 502- and 480-curves. Thus the form of the spectral sensitivity curve for the mechanism proposed here would not change greatly over a wide range of cone compositions. One would expect, however, that a true threshold sensitivity function would reproduce the 502-curve alone. Conversely, if the cones contained virtually only the 480-mμ pigment (for example, after prolonged pre-adaptation to light of long wave-length) one would expect the spectral sensitivity to follow the form of the broad-band 480-curve.

I gratefully record my thanks to Mrs. M. R. Bright for assistance with the experiments and to Miss C. Kerr for tending the squirrels.

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HOST SPECIFICITY OF TWO *XANTHOMONAS* SPECIES

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DYE[1] has stated that he was able to adapt cultures of twenty species of *Xanthomonas*, after four successive passages through the leaves of young dwarf bean plants, to produce infection symptoms on bean leaves that could not invariably be distinguished from those caused by *X. phaseoli* (Smith) Dowson. This finding suggests that the host specificity of the xanthomonads may not be a stable character.

An attempt was made to adapt *X. malvacearum* to produce infection symptoms on the leaves of *P. vulgaris* var. Banja, similar to those produced by *X. phaseoli* var. *fuscans* (*X. phaseoli* and *X. phaseoli* var. *fuscans* cause similar disease symptoms on *P. vulgaris* leaves). In the first experiment the plants were grown in the greenhouse until the first trifoliate leaf was half-developed. They were then placed in a humidity chamber maintained above 70 per cent relative humidity and between 22° and 30° C. for 24 hr. and afterwards inoculated by needle with pure bacterial slime taken from 48-hr. old cultures grown at 27° C. on potato–sucrose–agar plus chalk. The plants were covered for 24 hr. with a transparent polythene bag. No difficulty was experienced in re-isolating *X. malvacearum* from the vein tissue immediately adjacent to the points of needle puncture, 7–9 days after inoculation. Six single colonies were mixed to provide inoculum for re-inoculation. The process of inoculation, isolation and re-inoculation was repeated six times. On the 6th inoculation, leaves were also spray-inoculated by an atomizer with an aqueous suspension of the 'trained' bacterium from the 5th re-isolation until the leaves became water-soaked. As a control the leaves of another set of bean plants were sprayed with the original culture at approximately equal concentration (5×10^7 bacteria per ml.). The symptoms produced by the two cultures were similar, indicating that the successive transfers into bean tissue had not resulted in any alteration in its pathogenicity. Both bacterial cultures appeared to be capable of multiplying in bean tissue as the tissue of the sprayed leaves became slightly water-soaked in appearance 48 hr. after inoculation. After that time the tissue dried rapidly, forming light-brown necrotic areas. Both bacterial cultures caused, to the same degree, typical disease symptoms (angular leaf spot) when sprayed on to cotton leaves.

If the acquisition of new virulent characteristics is possible this must be engendered by some property or reaction of the new host plant and thus isolation and multiplication of the bacterium in pure culture is an unnecessary complication. In the second experiment bean leaves were used as the sole medium for multiplication of *X. malvacearum*. The technique was as follows: An aqueous suspension of *X. malvacearum* was prepared (approximately 5×10^7 bacteria per ml.) and sprayed by an atomizer on to the first and second trifoliate leaves (the second trifoliates were one-third fully developed) of 10 bean plants grown in the green-

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house. One half of each leaflet was sprayed until the tissue became water-soaked. Two to three days after inoculation disease symptoms became apparent as diffuse water-soaked areas. These did not increase in size and dried to form sunken, light-brown, necrotic areas (Fig. 1). One week after inoculation the necrotic areas from about 60 leaflets were cut out from the surrounding unaffected tissue and macerated in 150 ml. of distilled water. After standing for 3 hr. to allow the bacteria to diffuse out of the tissue, the suspension was filtered through muslin. The filtrate was used to spray the first and second trifoliate leaves of a second batch of bean plants. Some of the bacteria were lost by run-off during spraying, but a high proportion of the bacteria that had multiplied in bean tissue were re-inoculated into fresh tissue. The process was repeated seven times. The 'trained' bacterium was isolated, on solid media, from several necrotic areas which resulted from the seventh spray. Several single colonies were mixed together in distilled water to form a suspension (concentration of about 5×10^7 bacteria per ml.). An aqueous suspension of the original culture of approximately equal concentration was also prepared. The two were sprayed on to leaves of bean and on to leaves of resistant and susceptible varieties of cotton. Although the infection symptoms produced differed according to the host plant, the reactions of any particular host to the suspensions of 'trained' and 'untrained' bacteria were identical.

In an attempt to adapt *X. malvacearum* to breakdown the resistance of the cotton variety *BAR* 12/15 (carrying the bacterial blight resistance genes B_2 and B_{6m}), the same 'training' procedure was employed as that described for the second experiment. The process of inoculation and re-inoculation of an aqueous suspension of the bacterium into *BAR* 12/15 leaf tissue was repeated 15 times. During the 'training' period there was no suggestion of any change in the pathogenicity of the bacterium to *BAR* 12/15 leaf tissue. This was confirmed by spraying both 'trained' and 'untrained' bacteria in aqueous suspension of approximately equal concentrations on to the leaves of two resistant and two susceptible varieties of cotton, when again no differences were apparent.

It was also found that there was no change in the virulence of *X. phaseoli* var. *fuscans* in response to a 'training' period in cotton leaf tissue involving 4 re-inoculations carried out as described in the second experiment. The 'trained' bacterium also retained its pathogenicity to *P. vulgaris*.

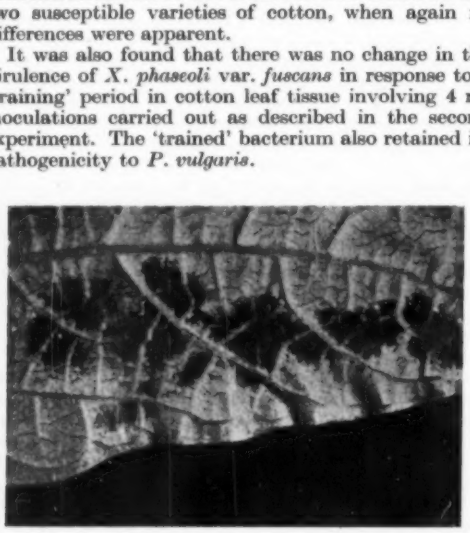

Fig. 1. The reaction of the bean leaf to either 'trained' or 'untrained' *Xanthomonas malvacearum* showing ill-defined, diffuse necrotic areas. (×2, lower surface)

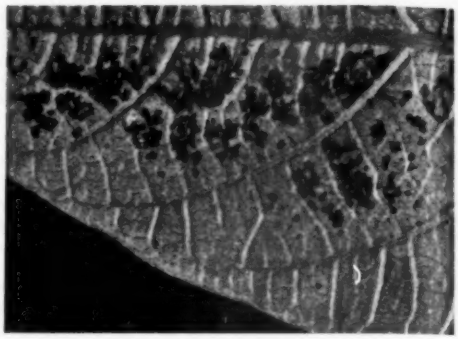

Fig. 2. The reaction of the bean leaf to *Xanthomonas phaseoli* var. *fuscans* showing well-defined, typical lesions. (×2, lower surface)

In these experiments both *X. malvacearum* and *X. phaseoli* var. *fuscans* multiplied in the tissue of either cotton or bean leaves. When both species were sprayed on to bean leaves at a concentration of 5×10^7 bacteria per ml. similar symptoms were apparent after 48 hr. Both species induced the tissues to become slightly water-soaked in appearance, *X. malvacearum* to a lesser degree than *X. phaseoli* var. *fuscans*. After this time the symptoms became markedly different. The areas of leaves sprayed with *X. malvacearum* dried rapidly, forming light-brown necrotic areas (Fig. 1), while those sprayed with *X. phaseoli* var. *fuscans* became progressively more water-soaked, resulting in typical angular leaf spots (Fig. 2). When the concentration in inoculum was reduced to 5×10^3 bacteria per ml., *X. malvacearum*, 'trained' and 'untrained', caused no symptoms to develop on bean leaves whereas *X. phaseoli* var. *fuscans* caused typical infection.

Thus, in these experiments there was no change in the host specificity of two cultures of *X. malvacearum* or in the one of *X. phaseoli* var. *fuscans*, a marked contrast to the evidence presented by Dye, in whose paper three important points are omitted. (1) He did not mention whether the change in virulence was sudden or whether there was a gradual increase in severity of infection symptoms. He only stated that on the fourth inoculation, lesions were allowed to develop for seven days and that a progressive disease was produced by all twenty species of *Xanthomonas*. (2) He did not compare his 'adapted' bacterium with the original culture. (3) He did not reverse the experiment, that is, attempt to adapt *X. phaseoli* to any one of the twenty species of host plant. It is thus difficult to see how he can claim that a change did in fact occur in any of the twenty *Xanthomonas* species.

Because a close physiological and biochemical relationship exists between many of the 'phytopathogenic species' of *Xanthomonas*[2], it is generally considered that many of these, which have been named after the original hosts, should be regarded as having the status of formae speciales[3], the differentiation of which is host specificity. If a further classification of the genus *Xanthomonas* is to be based on host reaction, the environmental conditions under which the host plants are grown, together with such considerations as the maturity of the host plant at time of inoculation, the inoculation technique or techniques, and the concentration of inoculum, must all be standardized.

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LETTERS TO THE EDITORS

PHYSICS

Resonant Scattering of the 14-keV. Iron-57 γ-Ray, and its Interference with Rayleigh Scattering

When radiation is scattered by a resonator, the question of its identity of frequency and coherence of phase with the incident radiation is not simply answered except for an infinitely narrow incident line, when the scattering is fully coherent (see, for example, the discussion by Heitler[1], written in the context of atomic resonances but equally relevant to nuclear ones).

Coherence may be experimentally proved by observing interference between the resonance radiation and some other form of scattering that is known to be coherent; for γ-rays, one would naturally look for interference with the Rayleigh (elastic electronic) scattering. In the long-studied 411 keV. resonance of mercury-198, such interference is unobservable[2], because of the thermal broadening of the line as well as for other reasons. It might just be observable in samarium-152, where the natural width of the 961-keV. line is not completely negligible[3,4] in comparison with the thermal; but the best conditions appear to be provided by the very narrow low-energy lines discovered by Mössbauer[5] in which a substantial part of the radiation is unaffected by recoil or thermal broadening.

After preliminary experiments in collaboration with Dr. B. S. Sood, in which the resonant scattering in metallic iron of the 14-keV. line of iron-57 was found to have the expected intensity, we decided that a foil of 65 per cent iron-57, electroplated on thin copper by the Isotope Division, Atomic Energy Research Establishment, Harwell, would give comparable amplitudes of resonant and Rayleigh scattering at convenient angles. We in fact chose an angle of $43 \pm 2°$, which would include the (211) Bragg reflexion. The geometry of the apparatus is indicated in Fig. 1: the source[6], about 20 mc. of cobalt-57 in a matrix of iron-56, was mounted on a 30 c.p.s. vibrator so that the exact energy of the emitted radiation could be oscillated through the resonant energy.

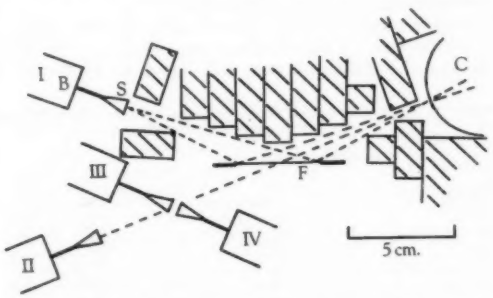

Fig. 1. Diagram of experimental arrangement. *B*, vibrator; *S*, source; *C*, proportional counter; *F*, iron foil. The shaded blocks are heavy alloy shielding. The different positions of the source and vibrator are: I, for scattering; II, for transmission; III and IV, for checking absence of shift in transmission

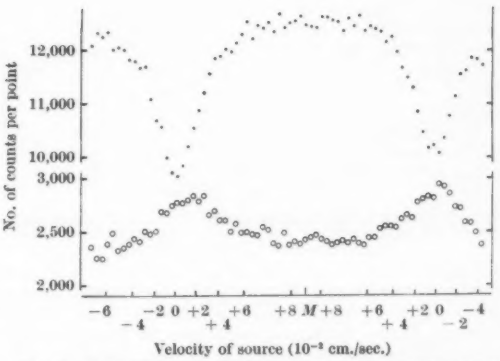

Fig. 2. Plots of intensity versus velocity for transmission (dots) and scattering (circles). The zero of the velocity scale is fixed only by the position of the transmission minima. Positive velocities are for the source approaching the foil. The position *M* on the velocity scale is the point at which the acceleration reverses

Auxiliary apparatus enabled the intensity of transmission through, or scattering from, the foil to be plotted automatically as a function of speed, the abscissæ of the resultant graphs extending over rather more than half a cycle of vibration, so that passages through zero velocity in both senses of acceleration were displayed.

Fig. 2 shows the transmission minima and scattering maxima obtained; it will be seen that the scattering peaks are somewhat broader and slightly nearer to one another than are the absorption minima.

The reduced separation of the peaks was, however, due entirely to the shift of one of them; presumably the whole pattern had suffered an instrumental drift during the long periods of operation that were necessary. We repeated the measurements with the apparatus set to display negative, instead of positive, speeds at the centre, alternating transmission and scattering measurements and adding the results of all sets of each kind. Fig. 3 shows that the peaks now appeared, as expected, more widely spaced than the dips, with no evidence of general drift. Thus, assuming the transmission dips to occur at exactly zero velocity, we can say that the maximum scattering occurs when the source is approaching the scatterer with a velocity corresponding to a fraction of the line-width.

It remains to verify that the dips correspond precisely to zero velocity; this need not be so if source and scatterer materials are not identical. This point was checked at the suggestion of Mr. D. A. O'Connor, by successive transmission experiments with the source assembly set in the positions marked III and IV in Fig. 1; this amounts to reversing the velocity-scale about the true zero. No change in the dip position was found.

If there is some coherence of phase between resonant and Rayleigh scattering, we should expect them to be in quadrature at exact resonance, moving towards coincidence of phase on the high-frequency side. Thus, on top of the constant intensity of the Rayleigh and the sharply peaked contour of the

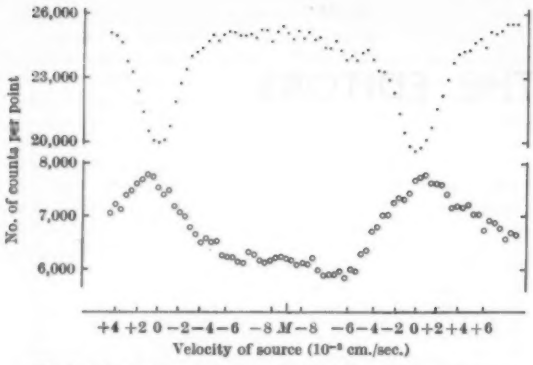

Fig. 3. Plots of intensity versus velocity for a separate experiment in which, as indicated on the velocity scale, negative velocities are displayed in the centre

resonant scattering, there should be added a dispersion-type curve representing the interference between them, with its maximum a fraction of a line-width to the positive side of zero velocity. The observed shift and broadening are probably to be explained in terms of such coherence, but the measurements are not yet detailed enough to show clearly the asymmetry of line-shape that is also to be expected or to determine what percentage of coherence exists; full coherence is not to be expected when the incident line is of similar breadth to that of the resonance.

We have also observed the resonant scattering from ordinary iron (2·2 per cent iron-57) at a mean angle of 90°, in the presence of a relatively large intensity of Rayleigh scattering. We found neither shift nor broadening of the scattering resonance, which is consistent with the expectation that, at this angle of scattering in a magnetic dipole transition, the resonant component will be polarized at right angles to the Rayleigh.

It is to be expected that the narrow-line resonance radiation will show, in its scattering from crystals containing iron-57, interference phenomena similar to those of X-rays. Interesting differences may arise from the resonant absorption which accompanies the scattering, from the narrowness of the line as compared with X-ray lines, and from the simple way in which the exact energy, and with it the phase of the resonant scattering, may be varied.

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Specific Fall-out Activity in Precipitation as a Function of Sampling Height

In 1956–57 Herbst *et al.*[1] found a definite increase with height in the fall-out activity in vegetation from an altitude of 800–1,000 m. and upwards. This was ascribed partly to the slow growth of the vegetation with increasing height and consequently greater accumulation of activity on it, and partly to greater fall-out at increased heights. Small[2] suggested in 1958 from examination of the variations in the natural activity of the air (radium B and C), compared with the meteorological conditions at ground-level, that during inversion conditions the vertical transport of bomb debris from above would decrease. By comparing the fall-out in a precipitation collector situated in the top of a 123-m. mast with that in corresponding collectors at ground-level, I found[3] for the period September 1958–March 1959 more fall-out in the former than in the latter.

It was therefore decided to examine the fall-out activity in precipitation collected in different heights. At Risö, a 123-m. meteorological mast has been built for micrometeorological measurements[4]. At heights of 0, 7, 23, 39, 56, 72, 96 and 123 m. I fixed, at a distance of 1 m. out from the mast, eight 1-litre polyethylene bottles each provided with a 154 cm.² polyethylene funnel. The bottles were changed on the first of every month.

After the bottles had been collected, the amount of rain-water in each was measured and strontium-, cæsium- and cerium-carrier was added. The content of each bottle was then analysed for strontium-90 and -89, cæsium-137 and cerium-144 by the method of Osmond *et al.*[5]. The activities are given in pc./m.², and in pc./l., and are traced to the middle of the month. All the isotopes were determined for each of the ten months March–December 1959, except strontium-89 which was too weak in activity in the last months of the year for a reliable determination. A table with all the results of the measurements is given in Risö Report No. 14 (ref. 6). Table 1 gives the annual average values for the specific activity at the different heights.

A regression analysis on the figures in Table 1 shows for all the isotopes an increase in specific activity with the height. Strontium-90 displays an increase in activity of 15 per cent per 100 m., cæsium-137 of 19 per cent, cerium-144 of 27 per cent and strontium-89 of 31 per cent.

A regression analysis of the results for the ratio strontium-89/strontium-90 (see Table 2) shows an increase in that ratio with height of 13 per cent per 100 m.

The present work shows that it is possible to detect a change in the specific activity in precipitation even within a difference in height of 100 m. (The increase in total fall-out with the height is still more significant, due to increasing amount of precipitation, when ascending[6].)

The reason for this difference in specific activity must be due to the varying contribution of dry fall-out in the different rain-collectors, as it would be

Table 1. Average Specific Activity in Precipitation for the Period March–December 1959

Height (m.) \ Isotope	Strontium-90 (pc./l.)	Strontium-89 (pc./l.)	Cæsium-137 (pc./l.)	Cerium-144 (pc./l.)
0	14·9	217	14·8	195
7	14·6	174	18·9	189
23	16·5	164	20·3	175
39	16·6	204	20·9	233
56	16·2	214	22·6	233
72	15·9	205	19·2	212
96	17·0	242	22·0	240
123	18·0	257	21·2	251
Mean	16·2	210	20·0	216

Table 2. Average of Ratio of Strontium-89 to Strontium-90 for the Period March–August 1959

Height (m.)	Ratio of strontium-89 to strontium-90
0	7·5
7	7·1
23	7·5
39	6·1
56	7·0
72	7·4
96	8·2
123	8·3

difficult to imagine how the rain could be less radioactive when falling down through the air.

(Regression analysis has shown that all the results are significant at the 1–2 per cent level except cæsium-137 and the ratio strontium-89/strontium-90, which are significant only at the 10 per cent level.)

At an altitude of 60 m. above the Risö area, there are on the average inversion conditions during 25 per cent of the time[4]. In the lower regions inversion is more frequent, in the upper less. In accordance with Small's results[2], it would be reasonable to suppose that these inversion conditions are one of the reasons why more dry fall-out is found in the bottles placed at the upper part of the mast than in those at the lower. Another factor contributing to the difference in activity will be the increasing wind velocity with increasing height[4]; this will give rise to a greater deposit of airborne particles in the higher bottles than in the lower ones[7]. Hence we get a higher specific activity in rain when the sample is taken at an altitude of, say, 100 m. than at ground-level.

Furthermore, it seems probable that short-lived fission products from fall-out would show a more pronounced variation with height than long-lived products. This is to be expected if one assumes that the dry deposit is of greater particle-size than the deposit from rain. Larger and heavier particles would be deposited more readily than fine particles[7], and consequently the fine particles will consist preferentially of older fission products than coarse ones. As a consequence of this, the age of the fall-out will decrease with increasing sampling height of the precipitation. As an example, the age determined in March 1959 from the strontium-89/strontium-90 ratio was 170 days at 0 m. and 148 days at 123 m. above ground-level.

I thank Mr. Heinz Hansen and Mr. Ulf Christiansen for many stimulating and inspiring discussions, especially concerning the statistical evaluation of the results.

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Radiative Transfer of Energy in the Core of a Heated Tube

While investigating the temperature distribution along a thin-walled tube heated by passing an electric current through it *in vacuo* we had naturally to calculate the radiative transfer of energy occurring in the hollow core of the tube[1]. Taking the tube to be a circular cylinder of diameter D, and considering an annular ring of width dx, the net gain in energy by the ring due to radiative transfer can be readily calculated. If the ring is not too near the ends, this is found to be equal to:

$$4\pi\sigma D^2T^2\,(dT/dx)^2\,dx + \frac{4}{3}\,\pi\sigma D^2T^3\,(d^2T/dx^2)dx$$

which may be expressed in the form:

$$\frac{d}{dx}\left(\frac{4}{3}\,\pi\sigma D^2T^3\,dT/dx\right)dx$$

where σ is Stefan's constant of radiation, and the emissivity of the walls is taken to be unity. It is as though the hollow core had a thermal conductivity of magnitude:

$$\varkappa_r = \frac{16}{3}\,\sigma DT^3 \qquad (1)$$

One may compare this with the expression obtained by Bosworth[2] for the radiational conductivity of a very hot gas, in which the photons emitted by the molecules, as distinguished from the molecules themselves, are regarded as diffusing into the gas, his expression being:

$$\varkappa_{rg} = \frac{16}{3}\,\sigma l_0T^3 \qquad (2)$$

where l_0 is the mean free path of the photons, which may be taken to be the reciprocal of the absorption coefficient of the gas for the photons. Indeed, the radiational conductivity of the hollow core may be regarded as the limiting case of the conductivity of a hot gas filling the core, when the pressure of the gas is reduced to zero and the free path of the photons is thus limited by the dimensions of the tube. In this limiting case the source of the photons will recede to the walls. Taking (2) to remain valid in this limiting case also, and comparing (1) with it, one can see that the mean free path of the photons in the hollow core is just D.

There is a close analogy between expression (2) for the radiational conductivity of a gas at high temperatures, and that for the lattice conductivity of a dielectric crystal at low temperatures, in which the transport of energy is by the low-frequency thermal elastic waves or phonons in the medium[3]. The closeness of the analogy arises ultimately from two factors: the upper limit to the wave number set by the Brillouin zone ceases to be of significance for these low-frequency waves, and, secondly, the frequencies are proportional to the wave numbers, as for the photons in a gas. These are also just the factors that lead to the T^3 law for the lattice specific heat.

The expression obtained by Casimir[3] for the lattice conductivity of a dielectric cylinder at temperatures low enough for the free path of the phonons to be limited by its diameter is analogous to that obtained here for the radiational conductivity of the hollow core—again as though the mean free path so limited is just D.

When the emissivity of the walls falls short of unity, one has to know how the fraction $(1 - \varepsilon)$ of the incident radiation that is not absorbed is returned to the enclosure. When it is in the form of specular reflexion, it is found that the thermal conductivity of the core is on this account increased by a factor $(2 - \varepsilon)/\varepsilon$, which is similar to the factor obtained by Berman, Simon and Ziman[4] in their expression for the lattice thermal conductivity of a cylindrical specimen at low temperatures, and also similar to the 'coefficient of slip' of a rarefied gas in its passage through a narrow tube[5].

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New Delhi. Aug. 23.

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Infra-Red Spectrum of Praseodymium Nitrite

In researches undertaken in this Laboratory, it has been necessary to characterize certain species in the thermal decomposition of a number of metal nitrates. In order to do this, it has been found useful to obtain the spectra of a number of metal nitrites. Nitrites are known to occur in the course of decomposition of a number of metal nitrates[1]; it has been found that praseodymium nitrite is a transient product in the thermal decomposition of praseodymium nitrate[2]. Praseodymium nitrite also appears as an intermediate in the interaction of nitrogen dioxide on praseodymia (Honig, J. M., and Vratny, F., unpublished work).

The majority of the nitrites used for comparison were obtained from commercial sources. Praseodymium nitrite was prepared by the method of Dutta[3] and Ferrari, Cavalea and Nordelli[4]. Stoichiometric quantities of praseodymium chloride and silver nitrite in aqueous solution were combined. The product was obtained by vacuum evaporation of the water. The viscosity of the ensuing solution, difficulty in crystallization, etc., strongly resemble the nitrate in character[5].

The spectra of the powdered metal nitrites were obtained on a double-beam Perkin Elmer model 21 spectrometer using sodium chloride optics. The spectra were obtained by dispersing the solid in 'Nujol' and by forming a potassium chloride pellet. The spectra were essentially the same except in the case of barium nitrite, in which case there appeared to be a shift in the 1,282-cm.$^{-1}$ band to 1,240 cm.$^{-1}$ in 'Nujol'. The use of the dual technique is recommended to assure absence of exchange, etc.[6].

The spectra of the simple nitrites: sodium, potassium, silver and barium, are rather straightforward and follow the characterization formulated by Williams[7]. The band at about 830 cm.$^{-1}$ represents the ν_2 bending mode, the band at about 1,250 cm.$^{-1}$ is the ν_1 symmetric stretching, and the band at about 1,360 cm.$^{-1}$ is the ν_3 asymmetric stretching. The

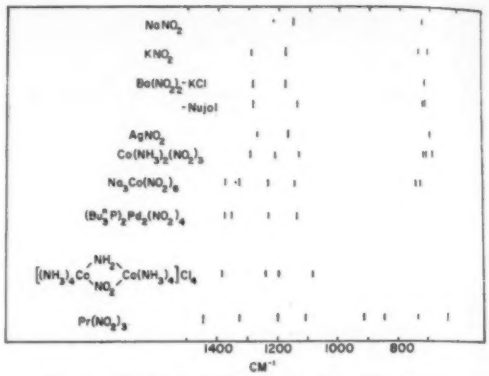
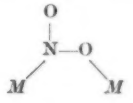


Fig. 1. Correlation diagram for various metal nitrites

splitting observed in the case of $Co(NH_3)_2(NO_2)_3$ occurs most probably in the ν_3 vibration and is configurational in origin. The spectrum of praseodymium nitrite is still more complex in that even greater splitting has occurred. The magnitude of the splitting is correspondingly greater than the field splitting observed in metal nitrates[8]. The distinction between nitro- and nitrite-linkage, as occurs with silver and sodium nitrite[9], is not a determining factor in this case. Recent work reported by Gatehouse[10] has shown that changes can be expected in the spectra of metal nitrites when the nitrite groups are arranged in either a *cis* or *trans* arrangement as in the case of $Co(NH_3)_2(NO)_2$, or when the nitrite group gives rise to a bridging structure, namely:



The spectra for two such compounds, reported by Gatehouse, are given in Fig. 1 along with other observed spectra. The two important modes of vibration for the bridging structure are:

at about 6·8 and 8·3μ, respectively. It can be seen that four characteristic bands occur in the high-energy region of the spectrum.

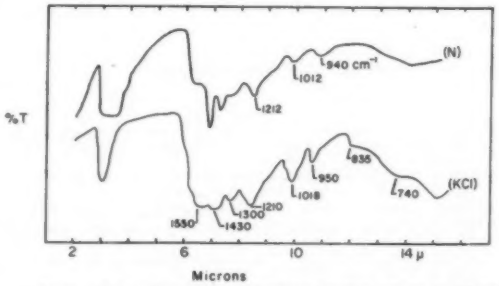


Fig. 2. Infra-red spectrum of praseodymium nitrite in 'Nujol' and potassium chloride pellet

The splitting produced in praseodymium nitrite strongly suggests a similar bridging structure. For purposes of comparing band intensities, the spectrum of praseodymium nitrite is reproduced in Fig. 2.

This work was supported by the United States Air Force Office of Scientific Research under contracts *AF*18(603)45 and *AF*19(604)5200 sub. 208.

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CHEMISTRY

Invariance of Molecular 'Inverse' Force-constants

In the quadratic form:

$$V = \tfrac{1}{2}\Sigma_1^n\Sigma k_{rs}q_rq_s \qquad (k_{rs} = k_{sr}) \qquad (1)$$

representing the harmonic approximation to the potential energy of a vibrating molecule, the 'force constants' k_{rs} depend on the definition of the internal co-ordinates q_i used to represent small changes in the molecular configuration. For example, a full analysis[1] of the vibrations of $^{14}NO_2$ and $^{15}NO_2$, with co-ordinates q_1 and q_2 the NO bond stretches, gives for the stretch and stretch-interaction constants $k_{11} \equiv k_{22}$ and k_{12} the following values (in units 10^5 dynes/cm.): (i) 10·884 and 1·9914 and (ii) 15·507 and 6·614 in the two cases where the third co-ordinate is (i) the ONO angle distortion and (ii) the change in OO distance. Thus the interpretation and comparison of force-constants obtained by different co-ordinate analyses present difficulties, which naturally increase with molecular complexity.

'Inverse' force-constants, h_{rs}, may be defined as the elements of the matrix $\{h_{rs}\}$ inverse to $\{k_{rs}\}$. A changed definition or choice of the internal co-ordinates q_r corresponds to a linear transformation, and the necessary invariance of V in (1) under this transformation implies that the h_{rs} can be transformed like the dyad or set of products q_rq_s. Thus if new co-ordinates $q_3, \ldots, q_n$ (linear combinations of the old $q_1, \ldots, q_n$) are chosen, leaving the old q_1 and q_2 as members of the new set, then the elements h_{11}, h_{12} and h_{22} are unchanged; and in general, the inverse force-constants corresponding to any unchanged co-ordinates are invariant. This can be verified with precision by using full sets of force-constants k_{rs} for NO_2 in cases (i) and (ii) above.

This invariance of inverse force-constants holds similarly for the potential energy in the vibrations of a single symmetry class, where the k_{rs} are symmetry-co-ordinate force-constants, and a partial change is made in the choice of symmetry co-ordinates.

It has often been found useful tentatively to transfer one or more force-constants k_{rs} from one fully analysed molecule to another containing a similar atomic group. The present considerations suggest that it might be more suitable to transfer the corresponding *inverse* force-constants h_{rs}.

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Re-assessment of the Electron-spin Resonance Splitting Constants of Several Well-known Free Radicals

The hyperfine splitting constants of the electron-spin resonance spectra of several well-known free radicals in solution have been re-measured recently. In some cases discrepancies with the values quoted in the literature have been found.

The particular case examined was the detection and identification by electron-spin resonance of a free radical produced during the thermal cracking of hydrocarbons, as the perinaphthenyl radical (it is hoped to publish details of this work in due course). It was found that the values of the splitting constants for the perinaphthenyl radical differed from those already reported[1]. In order to check the calibration of the magnetic field, the splitting constants of several well-known free radicals were measured, and the values obtained in the present work are compared with those reported in the literature in Table 1. The accuracy of the magnetic field calibration is confirmed by the agreement obtained for the last two free radicals listed. (Similar agreement has been obtained for several other free radicals.)

Table 1. Hyperfine Splitting Constants for Free Radicals in Solution

Free radical	Hyperfine splitting (oersted) This work	Literature	Ref.
Perinaphthenyl	6·30 ± 0·1	7·3	1
	1·82 ± 0·05	2·2	
DPPH *	8·9 ± 0·2	10	2
B and K †	11·9 ± 0·2	13·5 ; 11·0	2 ; 3
	5·9 ± 0·1	4·9 ; 5·7	4 ; 5
Coppinger's ‡	1·45 ± 0·05	1·0 ; 1·3	
p-Benzosemiquinone	2·34 ± 0·05	2·368 ± 0·001	6
		2·37 ± 0·01	7
Hydrogen atom	512 ± 10	505·4 ; 508·7	8 ; 9

* α,α'-Diphenyl-β-picrylhydrazyl.
† 1,3-Dimethyl-1-(phenylimineoxide)-butylidene-3-N'-phenyl-N'-oxynitrogen.
‡ 2,6-Di-*t*-butyl-4-(3,5-di-*t*-butyl-4-oxocyclohexa-2,5-dienylidene methyl)phenoxy.

In view of the potential use of electron-spin resonance for identifying unknown free radicals, the need for an accurate measurement of the hyperfine splitting constants cannot be emphasized too strongly. The apparatus used for measuring the magnetic field was relatively simple, consisting of a proton resonance probe and a *BC* 221 crystal-controlled frequency meter. In most cases the derivative electron-spin

resonance spectra were obtained and field markers superimposed on the recorder trace. In a few instances the spectra and the proton resonance signal were displayed on an oscilloscope and the hyperfine splittings measured directly.

I thank the Directors of "Shell" Research, Ltd., for permission to publish this communication.

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Hydrogen-bonding in Calcium-deficient Hydroxyapatites

Many calcium phosphates precipitated from solution give the X-ray diffraction pattern of hydroxyapatite but depart from the ideal stoichiometry, $Ca_{10}(PO_4)_6(OH)_2$, of this compound. Pure hydroxyapatite can be prepared only under hydrothermal bomb conditions[1,2], whereas the appearance of hydroxyapatites with low Ca/P ratio from aqueous preparations seems to be the rule rather than the exception.

Index of refraction, density, and X-ray diffraction data have shown that it is possible to have calcium ions missing randomly from certain positions in the hydroxyapatite structure[3]. To effect electrical neutrality, it was suggested that hydrogen bonds, in the proper proportion, were present between the oxygens of adjacent orthophosphate groups[3]. Evidence is presented here to confirm the presence of hydrogen bonds in a series of hydroxyapatites, with low calcium, in proportion to the missing number of calcium ions.

A series of hydroxyapatites deficient in calcium were prepared by methods outlined in an earlier publication[4], and examined by X-ray diffraction to make certain that each preparation was a single phase. The samples were analysed for calcium gravimetrically[5], and for phosphorus by a differential spectrophotometric method[6]. Average indices of refraction of the samples, obtained by the double-diaphragm method of oblique illumination[7], confirmed the X-ray and chemical evidence that these samples were calcium-deficient hydroxyapatites. Details of all physical and chemical analyses will be given in a later publication.

The samples all show an infra-red absorption band near 3,400 cm.$^{-1}$, which corresponds to a hydrogen-bonded O–H stretching frequency. Weighed mixtures of the various calcium-deficient hydroxyapatites and potassium ferricyanide were prepared and examined in a commercial infra-red spectrophotometer. Spectra were obtained from a pressed film of the sample held between two sapphire windows of a sapphire pressure-cell[8]. The base-line technique was used to calculate the molar absorbance from the absorption band. The hydrogen-bond content was determined quantitatively by comparing the absorbance at 3,400 cm.$^{-1}$ with that of potassium ferricyanide at 2,105 cm.$^{-1}$. The latter was used as an internal standard to correct for the unavoidable differences in thickness of film of the samples.

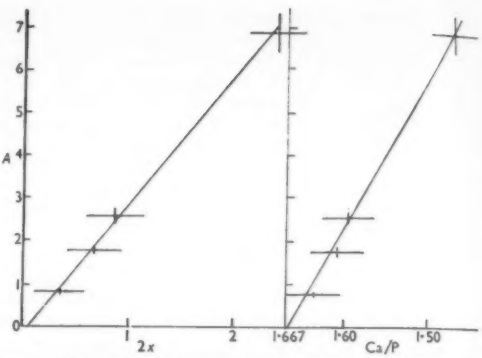

Fig. 1. Right. Plot of relative molar absorbance, A, at 3,400 cm.$^{-1}$ against the Ca/P molar ratio of a series of calcium-deficient hydroxyapatites

Left. Plot of absorbance values (A) against the number of gram atoms of H per mole (x) of calcium-deficient hydroxyapatite as calculated by theory (ref. 3). Standard deviations in ordinate and abscissa are indicated at each point. A was calculated by dividing the absorbance per mole at 3,400 cm.$^{-1}$ by the absorbance per mole of internal standard (potassium ferricyanide) at 2,105 cm.$^{-1}$

Fig. 1 shows that in a series of low-calcium hydroxyapatites examined, the infra-red absorption due to the hydrogen-bonded O—H group is inversely proportional to the Ca/P ratio. If the general formula for hydroxyapatite is assumed[3] to be $Ca_{10-x}H_{2x}(PO_4)_6(OH)_2$, it is then possible to plot the relative molar absorbance, A, against $2x$ (the gram atoms of hydrogen). Fig. 1 shows that such a plot is a straight-line function, indicative of the validity of the formula assumed.

The value of A approaches zero as the Ca/P molar ratio approaches 1·67, that is, the ratio for pure hydroxyapatite. Thus when the hydrogen-bond content is zero (that is, when $x = 0$), the compound is the end member, hydroxyapatite, which contains no hydrogen-bonding but only exhibits the O—H stretching frequency of free hydroxyl ion near 3,700 cm.$^{-1}$.

The hydrogen-bonded O—H stretching frequencies observed in the samples of calcium-deficient hydroxyapatites were all found to be near 3,400 cm.$^{-1}$. This indicates that the oxygen-to-oxygen distance of the O—H—O bonds did not change with varying hydrogen content. By means of the criteria of Lippincott and Schroeder[9], the probable O—O distance calculated from the O—H stretching frequency can be estimated at 2·85 Å. This is a reasonable distance between oxygen atoms of adjacent orthophosphate groups clustered around the three-fold axes of hydroxyapatite as calculated from the refined atomic parameters of this crystalline species[10]. It is not possible to say specifically from the present results which oxygen atoms are involved in the hydrogen-bonding. However, it is reasonable to assume that the hydrogen bonds will be as near as possible to the missing columnar calcium positions on the three-fold axes, since electrical neutrality will be maintained locally in a crystal.

This experiment has implications beyond the synthetic calcium phosphates discussed here. It has been suggested that the mineral portions of bone and tooth tissue are calcium-deficient hydroxyapatites[9,11]. The possible presence of hydrogen bonds in these materials may provide a much needed parameter for an estimation of the stoichiometry of these biological hydroxyapatites under varying conditions. In addition, there are many non-stoichiometric, inorganic systems in which hydrogen-bonding between electronegative ions may provide a balance between chemical perfection and imperfection due to missing cations.

This investigation was supported in part by Research Grant *D*–572, U.S. Public Health Service.

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The 1,2-Propylenediaminetetra-acetato-cobaltate(III) Anion

IT is implicit in a recent theory[1] of stereospecific effects in metal complexes containing optically active diamines that the optical isomers of complexes containing only one such diamine molecule should have exactly similar conformations.

To test this matter, the infra-red spectrum in the sodium chloride region of L-potassium (*d*-propylenediaminetetra-acetato)-cobaltate(III) trihydrate was compared with that of DL-barium (*dl*-propylenediaminetetra-acetato)-cobaltate(III) octahydrate. The spectra, in 'Nujol' mulls, were identical, as predicted by theory[1]. It is of interest that the infra-red spectra of *d*- and *dl*-propylenediamine tetra-acetic acids are not quite identical[2]. This was attributed to differences in hydrogen bonding.

A further point of interest is that the spectra of the cobalt complexes show a single strong carboxylate peak at 1,647 cm.$^{-1}$. This is good evidence for hexa-co-ordination by propylenediaminetetra-acetate, and is directly analogous to the single strong peak in the infra-red spectrum of the (ethylenediaminetetra-acetato)-cobaltate(III) anion (ref. 3). The spectra of complexes with copper(II), zinc and nickel show a splitting of this peak, presumably due to penta-co-ordination.

The cobalt complexes used were prepared by the methods given by Dwyer and Garvan[3]. Attempts to make complexes of formula $(Co(HPDTA)X)^-$, where PDTA is the propylenediaminetetra-acetate ion and X is the bromide or nitrite ion, by methods like those used by Schwarzenbach[4] in making the corresponding complexes of ethylenediaminetetra-acetate were unsuccessful. However, the reaction mixture from the attempted introduction of nitrite ion had a visible absorption spectrum which differed from that of the (propylenediaminetetra-acetato)-cobaltate(III) anion.

I thank Dr. G. D. Meakins for providing infra-red facilities and Dr. H. M. Irving for his help.

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BIOCHEMISTRY

Effect of Electrolytes on the Activity of Glutamic-Oxalacetic Transaminase

IN their method for the determination of glutamic-oxalacetic transaminase without precipitation of protein, Mohun and Cook[1] conducted transamination between aspartic and α-ketoglutaric acid to glutamic and oxalacetic acid in a phosphate buffer solution of *p*H = 7·5. Similarly, Yatzidis[2] used a 2 per cent solution of dipotassium hydrogen phosphate for incubation of the system previous to determining the enzyme activity in the serum.

Transaminase activity is known to be enhanced by iron Fe^{2+} and magnesium ions[3,4] and impeded by mercury and cyanide compounds[5]. Introduction of nickel, cobalt, aluminium and manganese ions was found to produce no variation in the activity of the enzyme[6].

As defined by Cabaud[7], the unit of transaminase activity is that of 1 ml. of serum giving rise to 1 μgm. of pyruvic acid in the system incubated. The activity of the serum investigated is determined by the use of a standard graph obtained from extinction measurements in hydrazone solutions of various pyruvic acid concentrations.

In the present investigation, which was carried out according to the method proposed by Mohun, the composition of the fundamental solution containing the substrate, that is, aspartic and α-ketoglutaric acid, was made to vary. In addition to the buffer solution, some electrolyte solutions as well as pure distilled water were used as solvents, simultaneously with, or instead of, the buffer. The experimental

Table 1

No.	Medium	Electrolyte introduced, in mgm. per sample	*p*H of system	SGO-T in Cabaud units
1	Phosphate buffer according to Mohun	9·4	7·5	22
2	Buffer–water (1 : 3)	2·4	7·5	33
3	Distilled water	—	5·0	34
4	Sodium chloride	8·2	4·8	25
5	Sodium chloride + (buffer–water (1 : 3))	10·6	7·4	24
6	Potassium chloride	9·5	4·8	29
7	Calcium chloride	1·4	4·9	43
8	Magnesium chloride	2·0	4·9	49

results are shown in Table 1, and prove that the activity of the enzyme increases as mineralization of the system decreases and acidity rises, attaining 34 Cabaud units in a medium of distilled water. The presence of sodium chloride and potassium chloride does not induce such activity. On the contrary, addition of calcium chloride or magnesium chloride to the solution incubated raises serum transaminase activity above the level obtained in an aqueous solution without electrolyte admixture, in spite of the relatively small amounts of calcium chloride and magnesium chloride introduced (greater amounts of calcium chloride provoke precipitation of sediment with substrate).

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Isocitratase as an Enzymic Indicator of Metabolic Pathways

Many micro-organisms form major quantities of *iso*citratase only when grown on substrates the catabolism of which ultimately produces acetate, and the provision of even small quantities of intermediates of the tricarboxylic acid cycle represses this formation of the enzyme[1–3]. This property can be utilized to decide between alternative modes of microbial degradation of growth substrates if one such alternative leads ultimately to the formation of acetate (or acetyl coenzyme *A*) and the other does not.

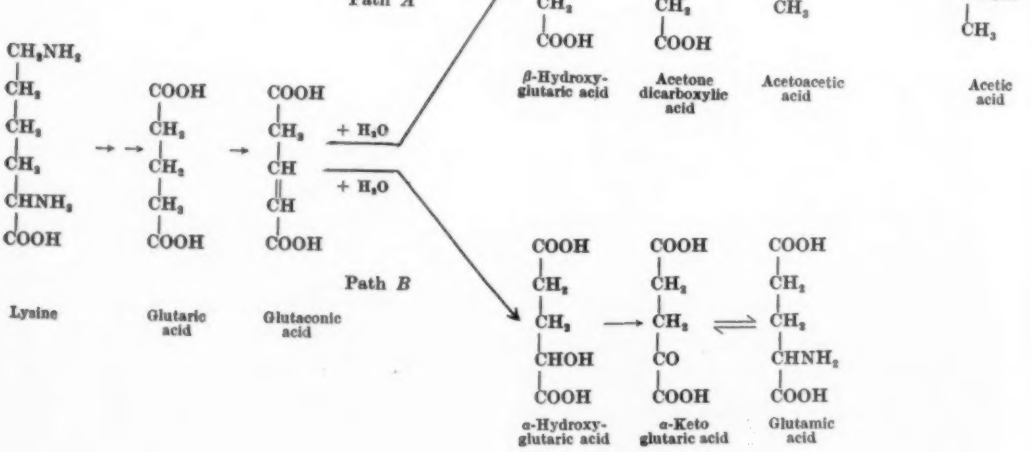
Table 1. *iso*CITRATASE ACTIVITY IN CELL-FREE EXTRACTS OF *Pseudomonas ovalis* CHESTER GROWN ON VARIOUS CARBON SOURCES

Carbon source for growth	Specific activity of *iso*citratase
Lysine	4·8
Glutaric acid	5·1
Acetone dicarboxylic acid	4·8
Acetoacetic acid	5·0
Acetic acid	5·2
α-Hydroxyglutaric acid	0·04
α-Ketoglutaric acid	0·05
Glutamic acid	0·05

Pseudomonas ovalis Chester was grown at *p*H 7·2 in 50 ml. of medium (ref. 1) containing 50 m*M* ammonium chloride, 50 m*M* phosphate, essential salts and the carbon source at 50 m*M* concentration. Extracts were prepared from the washed cells as previously described (ref. 1) and assayed for *iso*citratase (ref. 12). The levels of the enzyme in the extracts are expressed as the specific activity (μmoles of glyoxylate formed from *iso*citrate/hr./mgm. protein)

An example of this is the catabolism [illegible]. Studies with mammals and mammalian tissue preparations have established[4–7] that lysine is oxidized via pipecolic and α-amino-adipic acids, to glutaric acid. The subsequent fate of glutaric acid is [illegible] in some doubt. Evidence has been obtained for its β-oxidation to acetate[5,7–9], via acetone dicarboxylic acid and acetoacetate (path *A*), and its alternative α-oxidation[8,10,11] to α-ketoglutarate or glutamate via α-hydroxyglutarate (path *B*); the relative quantitative contribution made by these routes is not clear.

In an attempt to assess the relative importance of these two pathways in *Pseudomonas ovalis* Chester, the organisms were grown on media containing respectively lysine, or the sodium salts of glutaric, acetone dicarboxylic, acetoacetic, acetic, α-hydroxyglutaric, β-ketoglutaric and glutamic acids as sole sources of carbon. The *iso*citratase activity of extracts of cells thus grown was measured[12] (Table 1). Growth on the first five carbon sources listed in Table 1 (lysine and the substrates of path *A*) was accompanied by high and uniform levels of *iso*citratase, which indicates that their catabolism led chiefly to acetate. Growth on the last three substrates (the substrates of path *B*) led to formation of only traces of the enzyme.



Although these results do not provide evidence that the first five carbon sources listed are necessarily interrelated and are intermediates in the microbial degradation of lysine, they nevertheless show that the pathways of their catabolism are similar in leading to acetate, and different from the modes of degradation of the last three carbon sources. Since the specific activity of *iso*citratase after growth on lysine is closely similar to that produced after growth on postulated intermediates of its oxidation via path *A* and on the ultimate product, acetate, these results are consistent with its catabolism via this or some similar route also leading to acetate, and rule out path B as of quantitative importance in this organism. These findings are also in agreement with those of Andreoli and Wolfe[12], that oxidation of glutaric acid by an organism isolated from soil involves β-oxidation rather than α-oxidation.

We are grateful to Sir Hans Krebs for his encouragement and interest, and to Miss Patricia Lund and Mr. C. J. Iswale for their technical assistance. This work was supported in part by the U.S. Public Health Service, by the Rockefeller Foundation, and by the Office of Scientific Research of the Air Research and Development Command, U.S. Air Force, through its European Office, under contract No. *AF* 61 (052)–180.

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Estimation of Magnesium and Purification of Chlorophyll from *Chlorobium thiosulphatophilum*

Chlorobium thiosulphatophilum was grown under conditions similar to those described by Larsen[1]. '*Chlorobium* chlorophyll' (bacteriviridin) was extracted from the cells with 80 per cent methanol, transferred to ether, washed with water and dried over anhydrous sodium sulphate. The volume of the solution was reduced by evaporation under nitrogen, and the bulk of the chlorophyll precipitated by the addition of petroleum ether (b.p. 60–90). The chlorophyll was collected on talcum and washed with petroleum ether (b.p. 30–60) until the washings were colourless. The chlorophyll was removed from the talcum with acetone, transferred to ether, and washed with water. After drying, the solution was concentrated under vacuum, an equal volume of petroleum ether (b.p. 60–90) containing 4 per cent tetrahydrofuran added, and the solution adsorbed on to a sucrose column. The tetrahydrofuran was employed as a means of preventing precipitation of the chlorophyll in the column[2]. The column was developed by stepwise elution with *iso*propyl alcohol (0·75–2·0 per cent) in petroleum ether (b.p. 60–90°) containing 2 per cent tetrahydrofuran. The chlorophyll zone was cut out, dissolved in ether, and chromatographed as before. The absorption spectrum of the chlorophyll in ether was obtained on a Cary recording spectrophotometer. Peaks at 650, 575, 530, 425, and 406 mμ indicated that this chlorophyll was the '650' type described by Stanier and Smith[3], and that the organism studied was similar to or identical with *Chlorobium thiosulphatophilum*, strain *L*.

The magnesium content of the purified chlorophyll was determined spectrophotometrically by formation of a magnesium complex with sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido) - naphthalene-1′-(2-hydroxy-benzene-5-sulphonate)[4]. The purified chlorophyll, containing 0·5–10 μgm. of magnesium, was evaporated in a platinum dish, ashed with sulphuric acid, and collected as described by Koski and Smith[5]. The procedure was modified by keeping the total volume of water to less than 5 ml., and using 95 per cent ethanol for rinsing. The *p*H of the ethanolic solution of magnesium was adjusted to be slightly acid to phenolphthalein and transferred to a 25 ml. volumetric flask. Five ml. of the naphthalene-sulphonate reagent (0·15 mgm./ml. in 95 per cent ethanol) and 0·5 ml. of 0·08 *M* borax solution were added, the solution brought to volume with 95 per cent ethanol, and mixed. After 1 hr. the absorbance of the solution at 510 mμ was measured in a Beckman *DU* spectrophotometer using a distilled water blank and the concentration of magnesium in the solution calculated from a standard curve. The spectrophotometric sensitivity of this method is 0·0008 μgm. of magnesium/cm.[2] as compared with a sensitivity of 0·006 μgm. for the titan yellow method.

Assuming that there is a 1:1 molar ratio of magnesium and chlorophyll, the calculated molar extinction coefficient at 650 mμ in ethyl ether is $99{\cdot}7 \times 10^3$. Using the value for the specific extinction coefficient at 650 mμ obtained by Stanier and Smith[3] for '*Chlorobium*-chlorophyll-650' (113·5 l./gm. cm.) the calculated molecular weight of the chlorophyll was 879.

This work was carried out under the auspices of the U.S. Atomic Energy Commission.

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Tryptic and Phosphatase Action on Certain Synthetic Phosphorylated Peptides

With the development of a new procedure for the synthesis of O-phosphorylated peptides, in which di-*p*-nitrobenzyl phosphorochloridate is used as the phosphorylating agent[1], we have had occasion to study the action of alkaline and acid phosphatase on certain synthetic substrates.

A preliminary report of our results seems to be justified from the fact that a correlation between chemical structure and phosphatase action is strongly suggested. Moreover, additional information on the inhibitory spectrum of phosphoserine[2] was gained by the synthesis of α-carbethoxy-L-lysyl-L-leucyl-L-(O-phospho)serine and the action of trypsin on it.

So far as the latter case is concerned, the key intermediate, α-carbethoxy-(ε-carbobenzoxy)-L-lysyl-L-leucyl-L-serine benzyl ester (I), was prepared in a similar series of reactions used in the preparation of α-carbethoxy-(ε-carbobenzoxy)-L-lysyl-L-serylglycine benzyl ester[3]; the product had $[\alpha]_D^{29} - 19{\cdot}6°$ (*c.* 1 in glacial acetic acid). Analysis: calc., $C_{33}H_{46}N_4O_9$; C 61·6; H 7·1; N 8·7; found: C 61·6; H 7·1; N 8·6. Hydrogenolysis of it produced α-carbethoxy-L-lysyl-L-leucyl-L-serine (II) with $[\alpha]_D^{25} - 28{\cdot}5°$ as 1 per cent solution in glacial acetic acid.

Phosphorylation[1] of I and subsequent removal of protecting groups with palladized charcoal afforded α-carbethoxy-L-lysyl-L-leucyl-L-(O-phospho)serine (III), m.p. 245–250° (Kofler block), $[\alpha]_D^{25} - 24{\cdot}5°$ (*c.* 1 in glacial acetic acid.) Analysis: calc., $C_{18}H_{35}N_4O_{10}P$; N 11·2; P 6·2; found: N 10·9; P 6·1.

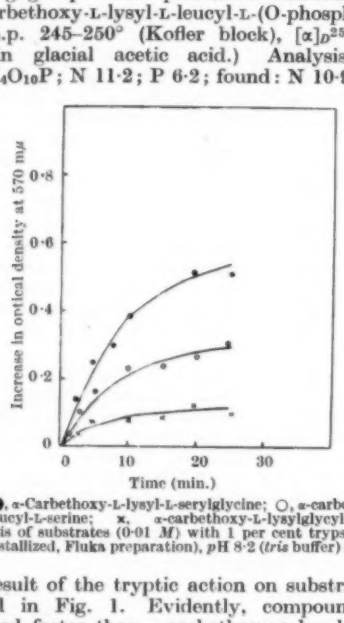

Fig. 1. ●, α-Carbethoxy-L-lysyl-L-serylglycine; ○, α-carbethoxy-L-lysyl-L-leucyl-L-serine; x, α-carbethoxy-L-lysylglycyl-L-serine. Hydrolysis of substrates (0·01 *M*) with 1 per cent trypsin (three times crystallized, Fluka preparation), *p*H 8·2 (*tris* buffer) at 25° C.

The result of the tryptic action on substrate II is indicated in Fig. 1. Evidently, compound II is hydrolysed faster than α-carbethoxy-L-lysylglycyl-L-serine (obtained from hydrogenolysis of α-carbethoxy-(ε-carbobenzoxy)-L-lysylglycyl-L-serine benzyl ester[1]) and slower than α-carbethoxy-L-lysyl-L-seryl-glycine It must be emphasized, though, that the colour yield given by the splitting products has not been taken into account; but, so far, only *iso*leucyl peptides are reported to give very low colour yield with the ninhydrin reagent[4].

Compounds II and III were incubated with trypsin at *p*H 8·2 at 25° and the incubation mixtures were then checked by paper chromatography at different intervals up to 2 hr. In contrast to II, compound III was extremely slowly attacked by trypsin. Only traces of the splitting product, L-leucyl-L-(O-phospho)-serine, could be detected. Even after 18 hr. when II was completely hydrolysed, compound III gave a faint spot at the position of L-leucyl-L-(O-phospho)-serine.

It might be of interest to note that the splitting of II by trypsin is not inhibited in the presence of III. Similarly, α-carbethoxy-L-lysyl-L-(O-phospho)-seryl-glycine (m.p. 260–262°, Kofler block) does not retard the hydrolysis of α-carbethoxy-L-lysyl-L-serylglycine, when both substrates, in the ratio 1:1, 2:1 and 3:1, are incubated with trypsin. Since no competitive inhibition was observed under these experimental conditions, it seems reasonable to assume that the resistance of the above-mentioned phosphopeptides towards trypsin is due to the intense electrostatic field which results from the vicinity of a strongly acidic (phosphoric acid) and a basic group (ε-amino group of lysine).

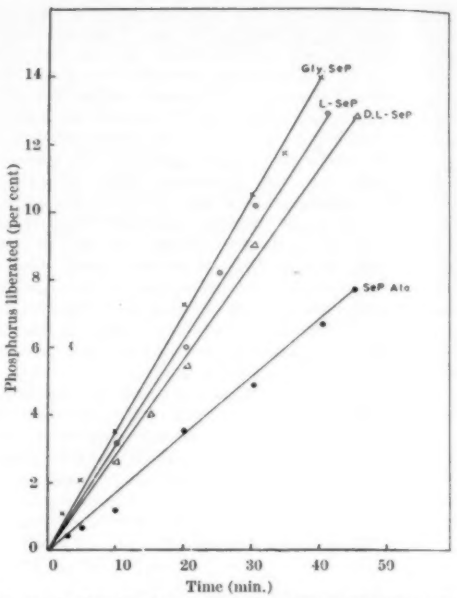

Fig. 2. Dephosphorylation of substrates (0·01 *M*) with alkaline phosphatase (0·1 mgm./ml., crude preparation, Fluka) in barbiturate buffer (0·2 *M*) *p*H 9·4 at 38° C. L-SeP = L-phosphoserine; D,L-SeP = D,L-phosphoserine

It should be recalled that L-(O-phospho)seryl-L-glutamic acid, isolated by Posternak and Pollaczek, was resistant to the dipeptidase action, but sensitive

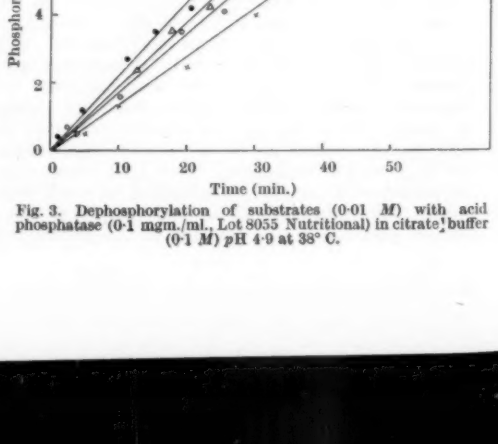

Fig. 3. Dephosphorylation of substrates (0·01 *M*) with acid phosphatase (0·1 mgm./ml., Lot 8055 Nutritional) in citrate buffer (0·1 *M*) *p*H 4·9 at 38° C.

to this enzyme after dephosphorylation[5]. Another tetrapeptide, Ileu.SeP.Val.Arg. was only slowly attacked by leucine aminopeptidase, while the hexapeptide, Lys.Glu-NH_2.Ileu.SeP.Val.Arg., readily yielded a mixture of lysine, glutamine, glutamic acid and a large amount of the tetrapeptide possessing an amino-terminal *iso*leucyl residue[6].

Provided that essential phosphopeptides are produced during the breakdown of caseins, one could assume that, in this connexion, the phosphoserine residues might play an important part in controlling the selective splitting of peptide bonds. Additional support to this assumption is offered by the phosphatase specificity observed with certain synthetic substrates tested so far.

It is apparent from Fig. 2 that alkaline phosphatase dephosphorylates glycyl-D,L-(O-phospho)-serine (Gly.SeP) considerably faster than L-(O-phospho)-seryl-L-alanine (SeP.Ala), calculated as the monohydrate.

Acid phosphatase, on the other hand, was found to dephosphorylate L-(O-phospho)seryl-L-alanine faster than glycyl-D,L-(O-phospho)serine. The amount of phosphorus liberated was determined by the method of Fiske and SubbaRow[7].

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Ubiquinone (Coenzyme Q) in Insects

Lester and Crane[1] found that while tissues of vertebrates contain mostly the Q_{10}-homologue of ubiquinone (coenzyme *Q*), certain insects, notably the house-fly, *Musca domestica*, and the cabbage white butterfly, *Pieris rapae*, are characterized by the presence of the Q_9 homologue, that is, a ubiquinone with a side-chain of 9, instead of 10, isoprenoid units.

In continuation of our previous work on quinone-dependent respiratory systems in insects[2,3], we made an analysis of ubiquinone in the hawk-moth, *Celerio euphorbiae*. The fresh tissue was saponified, extracted with ether, the extract washed with water, concentrated to dryness, and the residue dissolved in absolute ethanol. The absorption of ultra-violet light due to the ubiquinone present in the ethanolic solution showed a peak at 272 mμ; but we found it impossible to shift it to 291 mμ by reduction with potassium borohydride. In order to purify the material, the ethanolic solution was kept at −15° for 2 days, the precipitate which had appeared was removed, and the supernatant solution instilled on Whatman chromatographic paper No. 1, impregnated with 'Vaseline'. The chromatogram was developed in 95 per cent ethanol, and the paper area corresponding to the ubiquinone eluted with absolute ethanol. In this solution it was possible to demonstrate the characteristic shift of the absorption maximum from 272 to 291 mμ, which occurs after reduction.

In order to identify the ubiquinone homologue obtained from the hawk-moth, the chromatographic method of Page *et al.*[4] was used. However, instead of dimethylformamide, 95 per cent ethanol was employed for developing the chromatograms. In this way, it has been possible to achieve a much better separation of authentic samples of the Q_9 and Q_{10} homologues, which had been prepared from the yeast *Torula utilis*, and ox heart, respectively, using a modified method of Crane *et al.*[5].

In addition to the hawk-moth, *Celerio euphorbiae*, desiccated material from two other species of insects has been examined, namely, the pupae of *Sphinx pinastri* (Lepidoptera) and the adult insects of *Anatis ocellata* (Coleoptera). Just like the hawk-moth, the other two species have been found to contain the Q_{10} homologue of ubiquinone.

A detailed account of the experiments will be published in *Acta Biochimica Polonica*.

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The Epoxide Nature of the Carotenoid, Neoxanthin

In his extensive investigation of the xanthophylls of leaves, Strain[1] described a common, widely distributed carotenoid which he named neoxanthin. Neoxanthin has since been reported by other workers, and the evidence suggests that, together with lutein and violaxanthin, it is one of the three major xanthophylls of leaves[1,2]. Despite its common occurrence, however, all that is known of this pigment is due to Strain.

Neoxanthin is characterized by: (1) its tight adsorption on chromatographic columns of magnesium oxide or aluminum oxide, where it is found above lutein, violaxanthin, and flavoxanthin; (2) its characteristic absorption spectrum, similar in shape and position to violaxanthin, and with more pronounced fine structure than lutein; (3) its solubility—neoxanthin is readily soluble in alcohol but only sparingly soluble in petroleum ether. On partition between 60 per cent aqueous methanol and petroleum ether, the pigment is found almost entirely in the hypophase[1].

Goodwin and Jamikorn[3] reported the presence of neoxanthin in the green flagellate, *Euglena gracilis*. Recently we too have found a pigment in this species which has similar spectral and adsorptive properties to the neoxanthin described by Strain. Strain worked much with barley. We have therefore compared this carotenoid to neoxanthin isolated from barley (*Hordeum*).

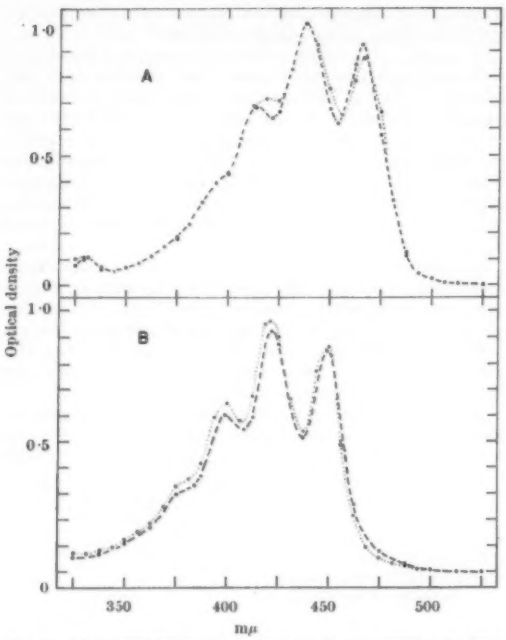
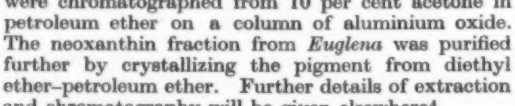


Fig. 1. (*A*) Absorption spectrum in ethanol of neoxanthin isolated from *Euglena* (dashed curve) and barley (dotted curve) (*B*) Absorption spectrum in ethanol of the furanoid oxide produced from neoxanthin in the presence of traces of dilute hydrochloric acid

The tissues were dried by grinding with anhydrous sodium sulphate and the pigments extracted with acetone and ethanol. After saponification, the carotenoids were shaken into benzene, leaving the chlorophyll in the aqueous hypophase. The carotenoids were chromatographed from 10 per cent acetone in petroleum ether on a column of aluminium oxide. The neoxanthin fraction from *Euglena* was purified further by crystallizing the pigment from diethyl ether–petroleum ether. Further details of extraction and chromatography will be given elsewhere[4].

Fig. 1*A* is the absorption spectrum of neoxanthin in ethanol. The major peaks of absorption are at 466 and 437 mμ, as compared with 467 and 437 mμ reported by Strain[1].

As shown by Fig. 1*B*, the addition of a trace of hydrochloric acid results in a hypsochromic displacement of about 18 mμ. This shift is characteristic of the isomerization of a single 5 : 6 epoxide (I) to the 5 : 8 furanoid oxide (II), with the removal of one double bond from conjugation[5].



(I) (II)

No separation of the pigments from the two sources could be achieved in a mixed chromatogram on a column of aluminium oxide (Merck) 'weakened' by the addition of 5 per cent water. The column was developed slowly with 40 per cent acetone in petroleum ether; 100 per cent acetone; 2 per cent and finally 4 per cent ethanol in acetone. With the addition of ethanol to the eluant, the carotenoid migrated slowly as a single band.

Table 1. Partition Coefficients of Neoxanthin

Source	Ratio of pigment in epiphase (petroleum ether) to pigment in hypophase (aqueous methanol): 60% methanol	50% methanol
Euglena	8:92	37:63
Barley	10:90	34:66

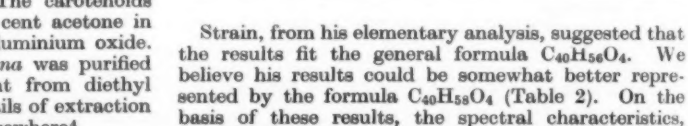
Quantitative partition coefficients, measured by the method of Petracek and Zechmeister[6], are given in Table 1. There can be little doubt that the pigment isolated from *Euglena gracilis* and neoxanthin isolated from barley are identical, and that this pigment is a mono-epoxide carotenoid. The degree of purification we feel accounts for the minor differences in absorption spectra.

From its spectral similarity to violaxanthin (ref. 1 and unpublished work by us) and neurosporene[7], this pigment appears to have only nine conjugated double bonds. Inasmuch as the epoxide group is in the 5:6 position, 5′:6′ must be saturated. Neoxanthin is much more hypophasic in partition tests than antheraxanthin (3,3′-di-hydroxy-5:6-epoxy-β-carotene)[4]. This and the firm adsorption during chromatography indicate the presence of a fourth polar group, such as a third hydroxyl.

Table 2. Elementary Analysis of Neoxanthin (data from Strain (1))

	Predicted for: $C_{40}H_{56}O_4$	$C_{40}H_{58}O_4$	Observed (average and range of four analyses)
C	79·93	79·70	79·37 (79·02–79·70)
H	9·41	9·69	9·67 (9·50–9·78)

Strain, from his elementary analysis, suggested that the results fit the general formula $C_{40}H_{56}O_4$. We believe his results could be somewhat better represented by the formula $C_{40}H_{58}O_4$ (Table 2). On the basis of these results, the spectral characteristics, behaviour on partition, and adsorption we propose the following tentative structure (III) for neoxanthin:



neoxanthin

(III)

Neoxanthin 3,3′,5′(or 6′)-tri-hydroxy-6′(or 5′)-hydro-5:6-epoxy-β-carotene).

It should be pointed out that this structure is a possible intermediate in the formation of the di-epoxide, violaxanthin.

It also appears likely that foliaxanthin, a mono-epoxide of unknown structure reported by Cholnoky *et al.*[8] as present in the leaves and fruit of the yellow paprika, is identical with neoxanthin.

This investigation was supported in part by a grant (*B*-2077) to one of us (T. H. G.) from the Institute of Neurological Diseases and Blindness, and a grant (*A*-2087) to the other (N. I. K.) from the Institute of

Arthritis and Metabolic Diseases, United States Public Health Service.

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A Method for concentrating Solutes of High Molecular Weight

CONCENTRATION of diluted aqueous solutions of labile solutes of high molecular weight, such as proteins, is often difficult in the laboratory as well as on a technical scale. We wish to present a mild concentration method, which seems to be useful in such cases.

This method is founded on the special property of a cross-linked dextran 'Sephadex' (Pharmacia, Uppsala (Sweden), which, even after swelling to a gel in aqueous media, will not admit large molecules, while there is little or no restriction of the diffusion of smaller molecules. If, therefore, dry 'Sephadex' is added to a solution, a concentration of solutes of high molecular weight is obtained corresponding to the amount of water taken up by the swollen grains. At the same time, salts and other solutes of low molecular weight are removed to an extent that ionic strength and *p*H remain approximately constant during the procedure.

As an example of the applicability, we wish to report an experiment where the liquid medium from a culture of *Polyporus versicolor* was concentrated prior to the isolation of cellulase. The purification of this enzyme will be described in greater detail elsewhere (Petterson, G., Cowling, E., and Porath, J.).

7·5 litres of clarified culture medium were divided into three equal portions and 750 gm. 'Sephadex *G*-25' were added to each of these. The sieve fraction of this material was 50–100 mesh and its water regain was 2·6 gm. per gm. of dry 'Sephadex'. The thick suspension was stirred for 10 min. to obtain complete swelling of the gel, after which the gel grains were separated from the concentrated enzyme solution in a basket centrifuge (*M.S.E.* 3000). The centrifugation was started with a moderate centrifugal field, which was progressively augmented as the flow decreased. The filter cake was then washed by spraying a small amount of water centrally. The centrifugates and washes were pooled and subjected to an additional concentration step. At this stage the volume of the solution was small enough to be handled in one operation. After a third treatment the volume was reduced to an extent which made further purification by traditional methods more convenient. The concentration in three steps was accomplished in less than 4 hr. in spite of the large number of operations. (It should be possible to reduce the time required to about 1 hr. by omitting the washings and by using a larger centrifuge in the first step.) The process was followed by determination of the cellulase activity as measured by the increase in the number of reducing end-groups[1]. The results obtained are given in Table 1.

Table 1. CONCENTRATION OF CELLULASE WITH 'SEPHADEX *G*-25'

	Enzyme solution (ml.) In	Out	Test quantity (ml.)	Activity (units per ml.)	Total activity
Original sample	7,500		1·0	0·532	4,000
First step					
Main fraction	7,500	3,045	1·0	1·245	3,790
First wash	300	300	1·0	0·400	120
Second wash	300	274	1·0	0·105	29
Second step					
Main fraction	2,930	1,130	0·5	3·816	4,310
First wash	117	122	0·5	2·196	268
Second wash	117	92	0·5	0·418	39
Third step					
Main fraction	1,020	410	0·1	8·48	3,480
First wash	41	34	0·1	2·58	88
Second wash	41	31	0·1	0·46	14
Third wash	41	26	0·1	0·19	5

The determination of activity is somewhat uncertain, as can be seen in the value for the main fraction of the second step. It is evident that this value is too high when compared to the original activity. According to the results in Table 1, the cellulase is almost quantitatively recovered in the 15-fold concentrated solution. The low activities in the washes show that it is possible to separate the concentrate almost completely from the swollen gel-grains quite easily.

For the application of the method described here, the following requirements must be fulfilled by the water-absorbing substance: (1) it should swell rapidly in water; (2) it should be easily and quantitatively separable from the concentrate; (3) it should be insoluble and not contaminate the concentrate; (4) it should not adsorb solutes which are to be concentrated.

Generally all these demands seem to be satisfied by 'Sephadex', if an appropriate particle size is used. Its upper limit is determined by requirement (1), and requirement (2) determines the lower limit. According to our own experience, only a small amount should pass a 100-mesh sieve.

Several procedures have been tried to separate the swollen gel-grains from the concentrate. The method described here, using a basket centrifuge, is particularly suitable for concentrating large volumes, while filter adapters in centrifuge tubes have been used when the concentration is carried down to a 1-ml. volume. The separation can, of course, also be made by filtration with suction or pressure; but losses are in general higher. Experiments have also been made to separate the concentrate in a column. For this purpose the suspension of gel in the concentrate is poured into a column and the concentrate is displaced by water. The method gave satisfactory results when the packing was carried out in such a way as to give a sharp boundary between the concentrate and the displacer.

Concentration with 'Sephadex' has been used for other substances sensitive to evaporation or freeze-drying, such as choline esterase, pancreatic lipase, phosphatases, and phosphodiesterases. Concentration of pepsin has been described by one of us[2]. In connexion with paper electrophoresis, this method has been used routinely.

Generally, very high recoveries have been obtained, amounting to more than 90 per cent at 10–20-fold concentration. In connexion with experiments involving some proteins of low molecular weight (cytochrome c and a phosphatase from *E. coli*), some complications have appeared. A considerable part of the activity remained in the gel and could be recovered only after extensive washing of the filter cake. Difficulties will probably arise also in the concentration of very viscous solutions.

It is of practical importance that the 'Sephadex' could be regenerated. This was done by first washing with water, followed by two suspensions of the material in ethanol, and drying at 80° C. The dried substance could then be used for new concentration experiments without any detectable difference in its properties.

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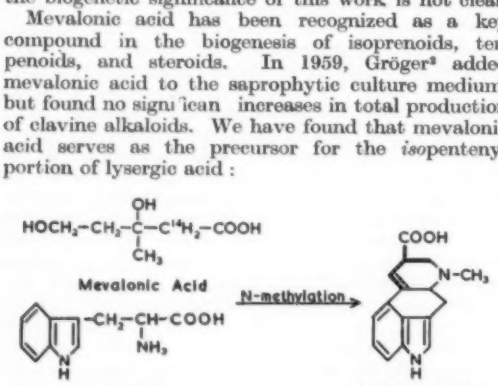
Biogenesis of Lysergic Acid in Ergot

Several schemes have been proposed for the biogenesis of the medicinally valuable lysergic acid moiety of the ergot alkaloids[1–5]. Recently, it was shown by Taber and Vining[6], and later verified by Baxter *et al.*[7], that tryptophan (β-^{14}C) serves as a precursor for lysergic acid. Gröger and co-workers[8] demonstrated that tryptophan (β-^{14}C) is also a precursor for the chemically related clavine alkaloids, which are produced by certain strains of ergot; but the carboxyl group of tryptophan does not enter the molecule. The nature of the precursor of the remaining portions of lysergic acid and of the clavine alkaloids has remained a puzzle. Gröger[3] demonstrated that, when acetate (1-^{14}C) was fed to saprophytic cultures of ergot, the radioactivity was incorporated into the clavine alkaloids. Since the alkaloids were not degraded, the biogenetic significance of this work is not clear.

Mevalonic acid has been recognized as a key compound in the biogenesis of isoprenoids, terpenoids, and steroids. In 1959, Gröger[3] added mevalonic acid to the saprophytic culture medium, but found no significant increases in total production of clavine alkaloids. We have found that mevalonic acid serves as the precursor for the *iso*pentenyl portion of lysergic acid:



So far as we are aware, this is the first demonstration of mevalonic acid serving as a biogenetic precursor for an alkaloid. (Since this communication was written, clavine alkaloids have been derived from mevalonic acid by Birch, A. J., *et al.*, *Z. Naturforsch.*, **15**, b, 141; 1960.)

A series of cultures of *Claviceps purpurea* (*PRL* 1578) were grown in batches of 40 ml. of liquid nutrient medium in 125-ml. flasks. After 15–30 days, 1 μc. of radiologically pure mevalonic acid-2-^{14}C (21·9 mgm. = 1 mc.) was added to each flask. Two weeks later the medium and the mycelium were separated by filtration, the medium made alkaline with ammonia, and the alkaloids extracted with chloroform. The mycelium was dried, finely powdered, and defatted with petroleum ether. The alkaloids were extracted with ammoniacal ether. The two alkaloidal extracts were evaporated to dryness, and the residues were dissolved in a mixture of chloroform and *iso*propanol (3 : 1); a portion was spotted on formamide-impregnated paper[9] and chromatographed (descending) with carbon tetrachloride/chloroform/benzene (7 : 2 : 1). In this solvent system the ergot alkaloids are separated well and in order of increasing R_F values: ergotamine, ergosine, ergotaminine, ergosinine, ergocornine plus ergocristine, ergocryptine, ergocorninine plus ergocristinine, and ergocryptinine; the water-soluble ergometrine and ergometrinine remain at the origin.

Ergotamine and ergosine were eluted separately from the carbon tetrachloride–chloroform–benzene–chromatogram with 50 per cent ethanol and hydrolysed according to the method of Jacobs and Creig[10]. The hydrolysate was chromatographed with butanol/acetic acid/water (4 : 1 : 1) on Whatman No. 1 paper. Autoradiograms showed the radioactivity to coincide exactly with the location of lysergic acid (R_F 0·40) as established from its fluorescence under ultra-violet light and the blue colour given by this indolic compound with Ehrlich's reagent. The behaviour of the compound was identical to that of authentic lysergic acid and to the behaviour of lysergic acid in the hydrolysate of pure ergotamine.

For the separation, identification, and isolation of ergometrine a second portion of the alkaloid mixture was chromatographed (descending) in butanol–acetic acid–water on Whatman No. 1 paper. In this solvent system ergometrine has an R_F of 0·60 while the peptide-type alkaloids have an R_F of 0·85. Both these fluorescent radioactive areas were eluted separately with 50 per cent ethanol, the eluate hydrolysed and chromatographed (descending) with butanol–acetic acid–water on Whatman No. 1 paper. In both cases the lysergic acid was found to be radioactive as revealed by autoradiograms. All the radioactivity of the alkaloids resided in the lysergic acid.

Mevalonic acid did not enter appreciably the general metabolism of the mould. More than 90 per cent of the radioactivity of converted mevalonic acid-2-^{14}C was found incorporated into the lysergic acid portion of the ergot alkaloids. The petroleum ether fraction of the mycelium contained less than 10 per cent of the total added radioactivity. The amino-acids which were extracted from the mycelium showed no radioactivity.

A more detailed and extended account of this work will be published elsewhere.

This investigation was supported by grants from Purdue Research Foundation *XR* fellowship No. 2255, and the National Institute of Health Contract No. *RG*–6606. We wish to express our appreciation to Dr. W. A. Taber and Dr. L. C. Vining, Prairie Regional Laboratory, Saskatoon, Saskatchewan, for the culture of *PRL* 1578, and to Dr. A. Hofmann,

Sandoz, Ltd., Basle, for samples of the pure ergot alkaloids.

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PHYSIOLOGY

Rectifying Properties of Heart Muscle

A FACTOR of likely importance in the genesis of the long-lasting action potentials of vertebrate heart muscle is the influence of membrane potential on the potassium permeability of the fibres. That a difference here exists between cardiac muscle and nerve is evident from Weidmann's[1] demonstration of a low slope conductance during the plateau of the action potential. But little information is so far available on the voltage dependence of the membrane conductance under conditions designed to avoid the generation of action potentials.

To study this point, so far as the technical limitations imposed by the structure of cardiac muscle at present allow, excised Purkinje fibres from sheep ventricles were used. While sharing the essential electrical properties of myocardial tissue, these fibres do not produce a mechanical response on depolarization; they are also insensitive to parasympathomimetic substances so that choline chloride or sucrose may be used as a substitute for extracellular sodium chloride to abolish excitability.

Fig. 1, *A* and *B*, shows how the membrane conductance of a Purkinje fibre in sodium-deficient solution depends on the direction and magnitude of the polarizing current. An outward current of 0·31 μamp., for example, produced the same voltage deflexion as is caused by nearly twice as great an inward current. Allowing for the cable properties of the fibre, this means that the conductance to a depolarizing current may be about four times less than to a hyperpolarizing current. Since the contribution of chloride ions to the membrane conductance of cardiac muscle is small[2], a decrease in the potassium conductance presumably occurs on depolarization. The direction of the potassium conductance change in Purkinje fibres is thus opposite to the predominant change in nerve[3], but has a parallel in skeletal muscle[4].

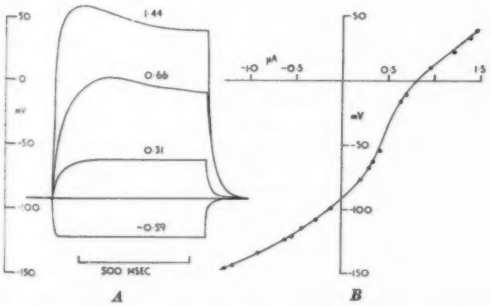

Fig. 1 *A*. Superimposed tracings of electrotonic potentials from a sheep's heart Purkinje fibre. Sodium ions in Ringer's solution replaced by choline. Depolarization is shown in an upward deflexion from a resting potential of −92 mV. Two closely spaced KCl-filled intracellular electrodes were used to record the membrane potential and to pass practically rectangular current pulses of a strength indicated by the figures on each record. *B*, Current-voltage relation for the same preparation. Ordinate, membrane potential at end of current pulse lasting 700 m.sec. Abscissa, total membrane current

With strong depolarizing currents a decline in the electrotonic potentials during the pulse may be observed, suggesting a gradual increase in conductance. This second effect probably accounts for the S-shaped relation between the amplitude of the electrotonic potential at the end of a long pulse and the polarizing current (Fig. 1 *B*), but over the physiological range the conductance remains below its value at the resting potential.

The slope conductance decreases appreciably over the range occupied by the slow diastolic depolarization in spontaneously beating preparations[1]. This alinearity may require consideration in interpreting the resistance changes observed during the pacemaker potential[5].

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Cardiac Action and Pacemaker Potentials based on the Hodgkin–Huxley Equations

SINCE the equations describing the nerve action potential were formulated by Hodgkin and Huxley[1], the range of phenomena to which they have been shown to apply has been greatly extended. Huxley[2] has applied them to the influence of temperature on the propagated response and to the repetitive firing observed in low calcium concentrations. More recently, Fitzhugh[3] has shown that the long action potentials induced by tetraethylammonium ions in squid nerve may also be reproduced.

The computations described in this communication were carried out with the aim of reconstructing the long-lasting action potential and pacemaker potential of cardiac muscle. Although this work was done independently, the results agree with those of Fitzhugh in showing that action potentials of long duration may be accounted for by Hodgkin and Huxley's formulation of the membrane properties. The description of the potassium current, however, differs from that used by Fitzhugh and provides a better description of the conductance changes.

The equations I have used to describe the sodium current are very similar to Hodgkin and Huxley's,

those for h (the variable describing the availability of sodium carriers) being based on Weidmann's[4] experiments on the availability of sodium carriers in Purkinje fibres.

The behaviour of the potassium battery in cardiac muscle differs from that in nerve in that the potassium conductance (G_K) falls when the membrane is depolarized[5]. A small delayed increase in conductance appears to be present when large currents are used, but this is not great enough for the conductance of the depolarized membrane to exceed the resting conductance. For the purpose of setting up equations to describe this behaviour, it is convenient to suppose that potassium ions move through two types of channel in the membrane. In one, G_K is an instantaneous function of the membrane potential and falls when the membrane is depolarized. A simple empirical equation has been used to describe the current in this channel. In the other type of channel the rectification is in the opposite direction and occurs with a delay. The conductance of this channel may be described by Hodgkin and Huxley's equations for delayed rectification with two modifications. First, the magnitude of this conductance must be small; accordingly the maximum conductance ($\overline{G_K}$) has been made less than 1/50 of that in nerve. Secondly, the time constants have been made very much longer to take account of the slow onset of the effect.

The membrane capacity was taken as 12 μF./cm.² (ref. 4), and the absolute values of the various conductances were adjusted to give a resting membrane resistance of about 2,000 ohms/cm.² (ref. 4).

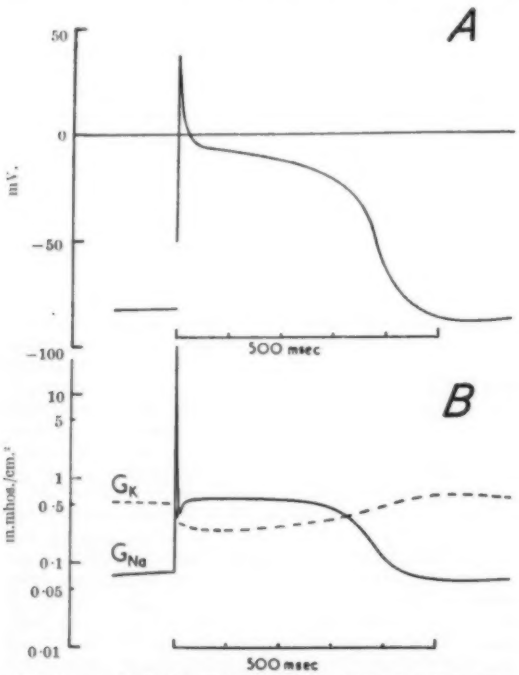

Fig. 1. *A*, Computed action potential. The integration was started by displacing the membrane potential to −50 mV., which is equivalent to a very short cathodal pulse of $3{\cdot}6 \times 10^{-7}$ coulombs/cm.². *B*, Time course of computed conductance changes on a logarithmic scale. G_K, potassium conductance; G_{Na}, sodium conductance. The potassium and sodium equilibrium potentials were set at −100 mV. and + 40 mV. respectively

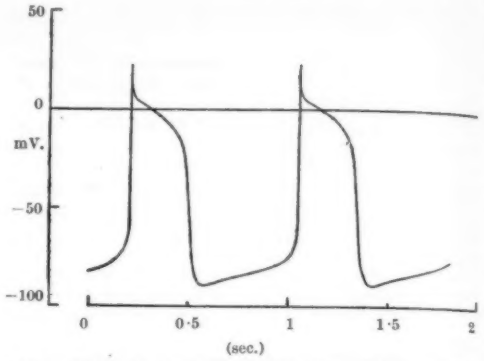

Fig. 2. Solution to equations in which the membrane potential is unstable in diastole so that pacemaker activity occurs. In this case, the potential at which the steady state sodium and potassium currents are equal and opposite is −38 mV. and an unstable state at this potential corresponds to a second solution to the equations

The equations were set up on a digital computer and were integrated by a numerical approximation using a step length of 0·05 m.sec. during the initial spike of the action potential and 0·3 m.sec. during the slower phases. In this connexion I should like to thank Dr. R. A. Buckingham, director of the University of London Computer Unit, for permission to use *Mercury* for this purpose, and Dr. M. J. M. Bernal and other members of the Computer Unit staff for advice on programming.

It was found that computed responses resembling action potentials of different durations could be obtained by inserting appropriate values for the constants in the equations for m (the variable describing the activation of sodium carriers). One of the solutions is shown in Fig. 1. It can be seen that the general shape of the action potential (A) corresponds very closely to that observed experimentally in Purkinje fibres[4]. The time-course of the computed conductance changes is shown in Fig. 1*B*. After an initial 'spike', the sodium conductance (G_{Na}) settles down to a value during the plateau which is about 8 times its resting value. G_K by contrast is below its resting value throughout the duration of the plateau.

The total membrane conductance ($G_K + G_{Na}$) rises during the plateau, but the slope conductance (determined by adding current to the equations at different times during the integration) was found to decrease. This is the result of a decrease in the sodium slope conductance which becomes negative towards the end of the plateau. Although the slope conductance during the plateau falls to a value which is equal to the resting conductance, the decrease was not so great as that observed experimentally[4]. This may be the result of a defect in my equations which over-estimate the potassium current produced by large depolarizations of the membrane.

If a large enough repolarizing current is added to the equations during the plateau, an all-or-nothing repolarization is initiated. The threshold for this phenomenon is about −30 mV. at the middle of the plateau, which is in fair agreement with that found experimentally by Weidmann[4].

In these equations there is always at least one potential at which the steady state sodium and potassium currents are equal and opposite. In the computations just described, this forms the resting potential and the system is stable unless excited. A

small change in the constants in the equations for m is sufficient to make the system unstable in diastole, and pacemaker activity then occurs. Such a solution is shown in Fig. 2. However, in order to bring about repolarization in this case a larger delayed potassium conductance had to be assumed. It is not yet known whether this is a necessary feature of the equations and further computations are being made to test this point. Nevertheless, the sensitivity of the computed pacemaker potential to changes in ionic conductance has been shown to correspond quite well with the experimental information available[6].

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Relative Vascularity of Certain Anatomical Areas of the Brain and Other Organs of the Rat

It need scarcely be pointed out that for the meaningful interpretation of the results of work where an exogenous material is administered to an animal and the assay performed on dissected areas of tissues with their content of blood, as is usually the case, it is frequently necessary to know the contribution of blood to the respective areas. This is particularly true if the concentration of a given substance is much greater in the blood than in the tissues.

Several approaches were made by different investigators[1–5] to determine the vascularity of the central nervous system of the cat, but so far as we know, nothing similar has been reported for the rat since the earlier work of Craigie[6]. He used the method of direct capillary counts of specimens from animals injected with carmin gelatin to determine the relative vascularity, but the limitations of this method are obvious. Furthermore, it has not included all the anatomical areas of interest to us. In 1955 Oeff and König[7] reported the blood content of several organs of the rat including whole brain in the non-bled out, bled out, and perfused animals using erythrocytes labelled with phosphorus-32. They did not, however, determine the blood content of the various anatomical areas of brain. In their work on the cat, Barlow *et al.*[1] have approached this problem using human serum albumin labelled with iodine-131 and demonstrated the relative vascularity by means of densitometric measurements of the autoradiograms.

Their technique consisted of killing the animal by intracardiac injection of a saturated solution of potassium chloride, dissecting out the brain, hardening it on a bed of dry ice followed by sectioning. The whole operation was performed at room temperature (29°). They observed that "tissue radioassay data of an anatomical structure within a single animal and of the same structure from animal to animal showed wide variation and were, therefore, useless for quantitative purposes. It is clear that dissection-sampling technique inadvertently included varying amounts of large blood vessels containing highly concentrated radioactivity giving rise to gross inconsistencies". However, by selecting areas on the autoradiogram corresponding to tissue areas containing blood within only small blood vessels and capillaries and avoiding large blood vessels, they were able to obtain values in good agreement with those found by others using capillary counting techniques. The problem referred to by Barlow *et al.* is a general one and arises also in distribution and metabolism work where such techniques are utilized.

It seems that the major factors which contribute to the inconsistencies in similar experiments are: (1) Varying amounts of residual blood that is retained in the brain tissue as a result of the varying loss which is inevitable in the process of dissection. (2) Contamination from blood which is also unavoidable. It is not difficult to see that in this case, where there is a tremendous concentration gradient from blood to the adjoining tissue, how adversely this situation can affect the results. (3) Since the operation is performed at room temperature, there is some condensation of moisture on the cold surface of brain slices which may lead to spreading, leaching, and further contamination of areas. The influence of these factors is greater when one is dealing with the very small areas of brain. In the present work, we have avoided these difficulties by using the following technique.

White male rats, weighing 250–300 gm., were injected by the tail vein with 10 μc./100 gm. of human serum albumin labelled with iodine-131 (*RISA*, Abbott). Pentobarbital sodium (4 mgm./100 gm.) was also administered along with the albumin to facilitate withdrawal of blood by intracardiac puncture, minimizing at the same time influences of stress on the animal. At the end of 15 min., 1 ml. of blood was withdrawn from the heart, and the animal was killed by rapid immersion in acetone–carbon dioxide mixture (−78°). It was then transferred to a refrigerated room (−10°), allowed to remain for 1 hr., and all subsequent operations were carried out there. The brain was chiselled out of the frozen animal with a 6 mm. wide chisel and after removal of the meninges and the larger blood vessels on the surface, 2–3 mm. thick serial coronal sections were obtained by free-hand sectioning with a razor blade. The areas dissected from the central nervous system were: cerebral cortex, cerebral white matter, caudate nucleus, thalamus, hypothalamus, hippocampus, inferior colliculi, cerebellar cortex, medulla and spinal cord. Similarly, sciatic nerve, kidney cortex, kidney medulla, periphery of the left lateral lobe of liver, skeletal muscle, and adrenals were also dissected. Of these, the cerebral white matter and the cerebellar cortex were the most difficult to dissect with any degree of assurance. It is to be noted that during the whole operation, in view of the fact that it is performed in the refrigerated room on the frozen animal, there was neither loss of blood nor contamination by blood; nor was there any spreading of activity from the blood as a result of condensing moisture. The areas dissected were homogenized in 1·5 ml. of water, 1 ml. of the homogenate transferred to weighed glass tubes and the radioactivity determined in a well-type scintillation counter. The tubes containing homogenate were then dried to constant weight in a desiccator. Table 1 shows the results from 15 animals. The results are expressed as a ratio of the specific activity of the area over the activity of a microlitre of hæmolysed blood of the same animal taken at the time of killing, times 10^2. An absolute determination of the vascularity was not attempted in this investigation, but it merely sought to establish a set

Table 1. Relative Blood Content of Different Areas of the Brain and Certain Other Organs of the Rat

Area	$\frac{\text{counts/min./mgm. dry wt.}}{\text{counts/min./}\mu\text{l. blood}} \times 10^2$
Cerebral cortex	8·49 ± 0·59
Cerebral white	3·33 ± 0·33
Caudate nucleus	5·53 ± 0·11
Thalamus	5·67 ± 0·13
Hypothalamus	3·61 ± 0·29
Hippocampus	5·38 ± 0·36
Inferior colliculi	8·32 ± 0·63
Cerebellar cortex	10·95 ± 0·68
Medulla	5·25 ± 0·35
Spinal cord	3·33 ± 0·33
Sciatic nerve†	3·37 ± 0·44
Kidney cortex	67·60 ± 3·40
Kidney medulla	140·70 ± 15·50
Liver	81·80 ± 4·03
Skeletal muscle	3·84 ± 0·34
Adrenals‡	117·20 ± 11·58

* Means of 15 experiments ± standard error.
† Means of 11 experiments ± standard error.
‡ Means of 4 experiments ± standard error.

of ratios which is adequate for the intended use of this information.

The experimental conditions for this work were carefully chosen so that identical conditions could be adapted for studies concerning drug distribution and permeability changes within the central nervous system. We are at present investigating two classes of compounds: one that enters the brain tissue rapidly and one that does not cross the blood–brain barrier, and it is hoped that the results will show the usefulness of the technique and the validity of the results obtained with iodinated albumin. It may be concluded from the present experiments that: (1) the vascularity of the central nervous system of the rat is certainly not uniform and the interpretations arrived at in the past on the basis of the average value of 0·024 ml. of blood per gm. of brain tissue[8] may need reconsideration. (2) There is a variation (for the range see Table 1) from animal to animal in the blood content of various areas. This would suggest the need for study of a large number of animals, particularly in the case of a substance the blood concentration of which is not much different to the concentration in tissue. (3) The results presented in Table 1 provide a reference scale which is of use in the study of distribution of drugs within the central nervous system of rat when the contribution of vascularity is also to be taken into account.

This investigation was supported in part by a research contract No. *AT*(11–1) 847 from the Atomic Energy Commission.

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Isolation of Glomerula from Rat Kidney

In contrast to the renal tubular system the glomerulum has not received as much attention biochemically as it deserves. This is mostly due to the difficulties in isolating a pure glomerular fraction. Only during the past ten years have a few methods been worked out which are, however, too complicated and time-consuming for the study of enzyme patterns in single normal or diseased animals. McCann's method[1], using manually isolated glomerula from frozen-dried sections, is limited by the small amount of tissue available for analysis. To obtain larger samples, attempts have been made to isolate the glomerula from crude kidney homogenate. Liberation of the glomerula from surrounding tissue was achieved by using a Potter–Elvehjem tissue homogenizer[2] with a mismatched plunger[3] or pushing the kidney through a fine-meshed wire gauze[4–8]. The purification of the glomerular fraction was performed either by repeated centrifugation[4], by filtration through a layer of glass balls[7], or by magnetic attraction following intra-arterial injection of iron trapped in the glomerula[8]. No detailed information on purity or yield of the glomerula was given. For enzyme work on normal and diseased glomerula a method had to be developed allowing rapid, and technically simple, isolation.

Table 1. Distribution of Alkaline Phosphatase in Fractions *H*, *M*, *T* and *G*

Fraction	*N*	μM *p*-Nitrophenol/min./mgm. protein	
		$\bar{X}$	*s*
H ('homogenate')	8	0·651	0·220
M (medulla)	7	0·030	0·015
T ('tubular material')	7	0·662	0·293
G (glomerula)	5	0·180	0·030

This was achieved by pushing out the glomerula of the surrounding tubules in a homogenizer with an ill-fitting pestle[2], centrifuging the resulting crude homogenate against an osmotic gradient and eliminating impurities by sieving several times through graded nylon mesh sieves. The capsule of kidneys of rats weighing 180–200 gm. is stripped off, the hilar structures and the medulla (Table 1: fraction *M*, 20 per cent w/v, homogenate in isotonic sucrose) dissected away. The remaining cortex is cut into small pieces with scissors, suspended in isotonic sucrose (final concentration 20 per cent, w/v) and coarsely ground in a Potter–Elvehjem apparatus[3] using an unmatched plunger until the tissue pieces have disappeared (fraction *H*). The suspension is then put through a nylon *HD* 250-μ mesh sieve (obtained from Züricher Beuteltuchfabrik A.G., Am Schanzengraben 15, Postfach 1123, Zurich 22). In the meantime, a plastic cylinder (Fig. 1,*b*) with a 100-μ mesh sieve as the bottom (*e*) was inserted into a centrifuge tube (*a*) filled with 25 per cent sucrose solution up to 2 cm. above the level of the sieve (*d*). 5–10 ml. of fraction *H* are layered on the hypertonic sucrose in the plastic tube (*c*), and centrifuged for 10 min. at 3,000 r.p.m. in a refrigerated centrifuge. There then appears at the top a yellowish viscous fluid containing microscopical particles which are believed to be of tubular origin because of their high content in alkaline phosphatase (fraction *T*). On the net are found small pieces of tubules and the majority of the glomerula. This material is resus-

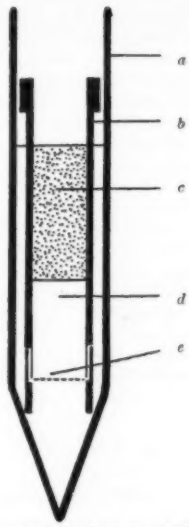

Fig. 1. Centrifuge tube (*a*) with plastic insert (*b*) for centrifugation through a mesh sieve (*e*) against an osmotic gradient (*c/d*)

pended in 8 per cent sucrose and filtered through a 150-μ sieve which eliminates most of the tubular fragments. The remaining impurities are removed by two or three more filtrations through 100–200-μ mesh sieves. A photomicrograph of the final glomerular fraction *G* is presented in Fig. 2 (courtesy of Prof. E. Hintzsche, director of the Anatomical Institute, University of Berne). Most of the glomerula are devoid of their capsule and only occasional specimens still show an intact Bowman capsule (right lower corner). It is important to carry out all the manipulations in the cold and to control the procedure by microscopic examination. Even so, the whole process can be completed within 1 hr. of killing the animals.

To determine the yield of glomerula, counts were made from fractions *H* and *G*. Assuming 27,000 glomerula per gm. rat kidney[9], 29 ± 8 per cent ($\bar{X} \pm s$; $N = 7$) of glomerula are recovered in fraction *H* and 7 ± 3 per cent in fraction *G*. This suffices for the assay of a number of enzymes and for manometric studies of the glomerula isolated from one animal. To obtain a quantitative measure of the purity of the glomerular fraction, alkaline phosphatase activity was determined[10] in homogenates and based on protein content[11]. The results (Table 1) reveal a three-fold concentration of this enzyme in fractions *H* and *T* as compared to fraction *G*. Work on a number of other enzymes, oxygen uptake and glycolysis of isolated glomerula will be presented elsewhere.

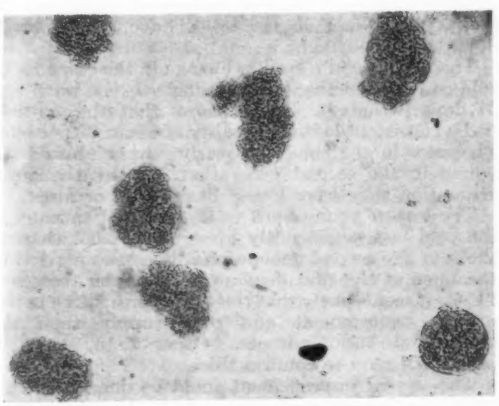

Fig. 2. Photomicrograph of fraction *G*

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Active Transport of Sodium by Isolated Frog Skin in Relation to Metabolism of Acetate labelled with Carbon-14

Although it is well established that isolated frog skin uses energy for the active transport of sodium, little is known about the metabolic processes providing this energy. Huf[1] showed that monobromacetate caused a decrease in potential across the frog skin which could be reversed by pyruvic and lactic acids, but not by glucose. Similar results were obtained by Francis and Gatty[2].

These experiments suggest, but do not clearly establish, that exogenous metabolites can be used by the skin. The use of appropriate metabolites labelled with carbon-14 appeared to offer an approach to this problem. Preliminary work by one of us (K. Z.) done in the Department of Biophysics, Purdue University, indicated that the isolated frog skin could take up and metabolize a number of carbon-14-labelled compounds.

The short-circuiting technique of Ussing and Zerahn[3] was employed for determining sodium transport. The apparatus used was only slightly modified from that of Zerahn[4]. The skin was placed as a barrier between two glass compartments so that each could be individually filled with solution, stirred, drained and refilled with ease. The compartment facing the inner side of the skin was filled with Ringer solution to which was added the labelled metabolite to be studied, and vasopressin to obtain a higher rate of sodium transport. Streptomycin was also added to this solution in about half the experiments to reduce bacterial growth.

In other experiments, it was found that isotopic equilibrium is not attained in several hours of equilibration. A preliminary 2-hr. period of contact of the skin with labelled metabolite was therefore allowed, and the bathing solutions of this period were discarded. The medium from subsequent experimental periods was acidified and the released carbon

dioxide collected in a small volume of sodium hydroxide using a spiral absorption apparatus developed by Holm-Jensen (Holm-Jensen, J., unpublished work).

The radioactivity of carbon dioxide samples was compared to the radioactivity of the inside solution and thus permitted calculation of the amount of tracer metabolite utilized.

The labelled metabolites were obtained from Amersham and were generally used at a level of 0·5 μc. per period. Acetate was chosen as a representative metabolite and was used at a level of 1 m*M*.

During the transport period the compartment on the outside of the skin was filled with Ringer solution. In this way the sodium transport can be determined from the reading of the short-circuit current. Total production of labelled carbon dioxide and thus total labelled acetate utilization were determined.

To determine the amount of the radioacetate used which did not contribute to sodium transport, a blank period of non-transport was done during which the outside Ringer solution was replaced with distilled water. Other means of obtaining non-transport periods, such as the use of an applied potential of 150 mV. (inside positive), gave similar results.

Alternate 90-min. periods of non-transport and transport were carried out, and the average value of acetate carbon-14 used for two non-transport periods was subtracted from the value of the intervening transport period. The labelled metabolites contained some labelled carbon dioxide. No correction was made for this activity because it represented a minor fraction of the labelled carbon dioxide collected, and furthermore, calculation of the difference as above allows for the presence of a blank.

The following assumptions are made in the calculations: (1) Both the carbons of radioacetate contribute to production of carbon dioxide to about the same extent. This was confirmed in work with acetate-1-^{14}C and acetate-2-^{14}C. (2) For each mole of acetate metabolized, two molecules of oxygen are utilized. Since Zerahn[4] and Leaf and Renshaw[5] have shown that one molecule of oxygen is used for each 18 atoms of sodium transported, each mole of acetate used for transport will provide for the transport of 36 atoms of sodium. Each atom of sodium will thus require the use of 1/36 mole or 0·0278 mole of acetate. (3) From the net acetate used and the amount of sodium transported, the fraction of the sodium transport due to the use of the acetate of the medium is calculated.

The experimental findings are summarized in Table 1. It is clear that the added acetate can be utilized by the isolated frog skin as is indicated by the first two columns and that it is utilized for sodium transport is shown by the third column. The fraction of the sodium transport supported by the metabolism of the exogenous acetate is given in the last column. (This is, furthermore, the relative specific activity in per cent of the radioacetate added.)

Table 1. Metabolism of Acetate-1-^{14}C by the Isolated Frog Skin

μmole/hr. of labelled acetate used during			Δ Sodium μmoles	μmole acetate per μmole sodium transported	Fraction of sodium transport by radioacetate (per cent)
Transport	No transport	Net			
0·216	0·139	0·077	5·8	0·0133	48
0·284	0·226	0·058	11·2	0·0052	19
0·442	0·226	0·216	15·0	0·0144	52
0·392	0·321	0·071	5·7	0·0125	45
0·466	0·329	0·137	10·5	0·0130	47
0·299	0·150	0·149	13·2	0·0113	41
0·344	0·180	0·164	13·2	0·0126	45
0·467	0·220	0·256	8·4	0·0304	109
0·312	0·165	0·147	11·8	0·0125	45
0·468	0·250	0·218	13·2	0·0165	59
0·386	0·203	0·183	12·3	0·0149	54

In similar work we have shown that lactate is used at a rate similar to acetate, but that glucose was metabolized to a smaller extent.

It can be concluded that: (1) The serosal surface of the isolated frog skin is permeable to exogenous acetate and lactate; (2) the skin can use these compounds for metabolism; (3) added metabolites can support sodium transport; (4) when the exogenous acetate is present at a level of 1 m*M*, an appreciable fraction of the total sodium transport can be directly related to the use of the added acetate.

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An Adaptive Change in an Inbred Mouse Strain maintained in a Cold Environment

A strain of *A2G* mice (*Mus musculus* L.), maintained for a number of generations in a room kept at −3° C., has progressively improved in ability to cope with the cold. The mice were obtained from Glaxo Laboratories, and had a long history of sib mating. When the breeding was begun in the cold environment, a parallel colony was started at 21° C. (controls). Members of a single litter were used to start the two colonies: one pair was kept at 21° C. and the other transferred to −3° C. These initial pairs were the parents of the first generation of mice studied. All subsequent breeding pairs too were litter mates, and were selected at random at the age of 5 weeks. Reproductive performance was recorded to the age of 28 weeks. There were few barren pairs in the cold, and only one after the fourth generation. One control pair, in the fourth generation, failed to produce young. The methods used, and some of the physiological changes in the mice kept in the cold, have already been described[1–3,5].

The evidence of a secular change in the response to cold comes from the loss of young between birth and 21 days (weaning). Fig. 1 shows that the controls had a consistent infant mortality of about 22 per cent. The mice in the cold lost nearly 80 per cent of the young in the second generation, but by the ninth generation they were losing 10 per cent or fewer.

The loss of young born to first-generation pairs in the cold was significantly lower ($P < 0.05$) than to those of the second generation. Since the parents of the mice of the first generation had been reared at 21° C., it seems likely that these mice provided a better uterine environment and milk supply than the second-generation parents. Other data, not yet published, tend to confirm this.

The secular improvement could be due to changes either in maternal performance or in the young; both could be involved. There was no evidence of a pro-

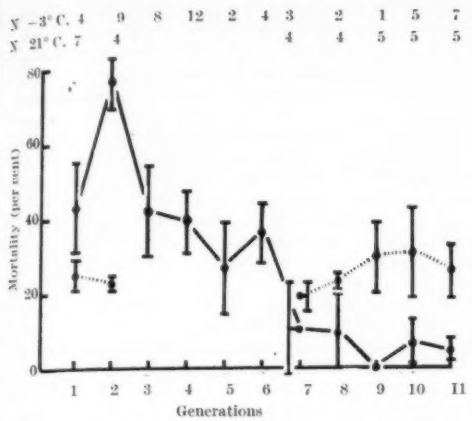

Fig. 1. Mortality between birth and 21 days, by generation. Continuous line: mice at −3° C.; broken line: controls. Each point is a group mean for pairs of one generation. Vertical lines represent standard errors. The numbers of pairs are shown at the top. No control data are available for generations 3–6

gressive increase in fecundity in the cold: in fact, the number of young born per pair did not change significantly in either temperature. The most probable sources of the improvement are (*a*) increased milk yield[6,8], (*b*) superior ability of the young to respond to the low temperature by increased heat production[3,4].

Whatever the physiological basis, the results require explanation in terms of either (1) a steady genetical change in the colony maintained at −3° C.; or (2) a cumulative maternal effect.

(1) The first hypothesis implies that the original strain was genetically heterogeneous despite its long history of sib mating (since mutation cannot account for a rapid progressive trend of the kind observed). If this were so, it would be necessary to assume that there were present genetical mechanisms which tended to maintain heterozygosis in the face of rigorous inbreeding. There are empirical grounds for rejecting this hypothesis, in the work of Deol *et al.*[7]: these authors give evidence that inbred mouse strains are genetically homogeneous, and that the formation of sub-lines is due to the occasional appearance of mutant genes which "tend to be fixed promptly in homozygous condition". The fact that the improvement continued even when the infant mortality was low is also against this hypothesis, since natural selection can occur only if the breeding of some members of each generation is diminished either by death or by reduced fertility.

There are also theoretical grounds for doubting whether heterozygosis at several loci could be maintained in an animal such as the mouse, since an excessively high loss of progeny would be required in each generation[9].

(2) The second hypothesis implies that the change is wholly one of 'nurture'. Survival in the cold demands extensive physiological adaptation. The young not only must themselves respond to the cold environment, but also may be influenced by the adaptive adjustments made by the mother: this effect could be through the environment either in the uterus or in the nest (the nest environment includes the milk supply). The hypothesis requires that the ill-effects of rearing the females in the cold were progressively reduced through a cumulative improvement in physiological adaptation to cold. There is no obvious argument against this, but there is little evidence to show how the change could have been brought about.

Neither hypothesis accounts for the fact that, from the seventh generation on, the infant mortality in the cold was consistently lower than in the warm. This implies that, in the long run, so far as survival during the first 3 weeks was concerned (though not in other ways), the cold environment was the superior one. Yet the infant mortality in the warm was similar to that reported by other workers; there is consequently no reason for thinking that there was anything wrong with the conditions in the warm room.

Whether the change in infant mortality was due to heredity or environment, it has implications for any experiments in which mice (and perhaps other mammals) are maintained for several generations in an environment very different from that of a control group. Just what these implications are depends on the exact nature of the change; this is being studied. The results will in any case be more fully reported and discussed later.

The work is supported by the Medical Research Council.

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Presynaptic Nature of Neuromuscular Depression in the Frog

When a number of successive nerve impulses arrive at a curarized neuromuscular junction, the induced end-plate potentials show a gradual decrease in size (Wedensky-inhibition, or junctional fatigue). Two different mechanisms, though not exclusive of each other, are conceivable.

(1) The amount of acetylcholine liberated at the nerve endings might be decreased, presumably in consequence of the depletion of the store of transmitter substance[1–4].

(2) The sensitivity of the end-plate to acetylcholine might be decreased as a result of the desensitizing action of the transmitter.

Recently, Thesleff[5] reported that the latter mechanism was a main cause of the diminution of the size of the end-plate potential in the rat. In the following experiment it will be shown that, in contrast with the case in the rat, the first mechanism is the main cause of neuromuscular depression in the frog and that the latter mechanism plays only a negligible part, if any.

A recording micro-electrode was inserted at the end-plate region of a curarized sciatic-sartorius preparation of the frog. Constant quantities of acetylcholine were applied electrophoretically from a second micropipette containing a 1·1 *M* solution of the substance, which was located on one of the sensitive spots of the same end-plate. End-plate potentials were induced

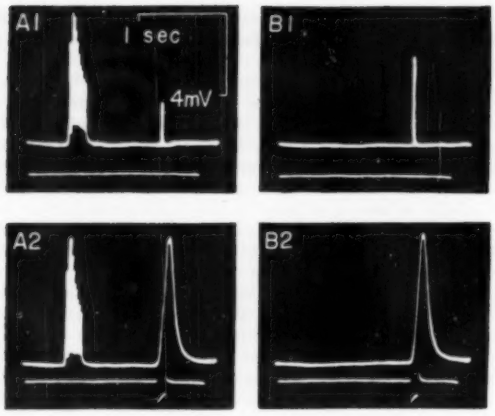

Fig. 1. End-plate and acetylcholine potentials following the conditioning repetitive stimulation. In each record, the upper trace shows the membrane potential recorded from the end-plate region. In the lower trace the strength of the current through the acetylcholine pipette was monitored.
A, End-plate potential (*A*1) and acetylcholine potential (*A*2) about 0·9 sec. after repetitive stimulation.
B, End-plate potential (*B*1) and acetylcholine potential (*B*2) 11 sec. after repetitive stimulation.
Conditioning stimuli were given every 20 sec. Neuromuscular transmission was blocked by *d*-tubocurarine 3×10^{-6}. The muscle was stretched by about 20 per cent of its resting length. Resting potential was 89 mV.
Monitor calibration: '4 mV.' scale = $1{\cdot}2 \times 10^{-7}$ A.

by supramaximal nerve volleys. End-plate and acetylcholine potentials were recorded at various intervals after the conditioning stimuli consisting of 10 successive nerve volleys at a frequency of 50 per sec.

Fig. 1 shows that about 0·9 sec. after the conditioning stimuli the size of end-plate potential is decreased by 55 per cent compared with that of the end-plate potential which is not preceded by repetitive stimulation. The amplitude of acetylcholine potentials, on the other hand, remains completely unchanged whether preceded by conditioning stimuli or not.

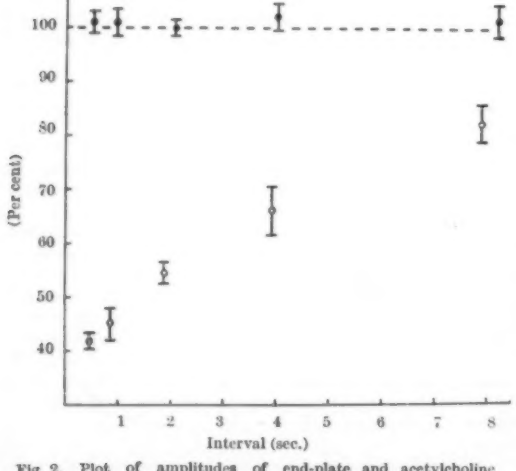

Fig. 2. Plot of amplitudes of end-plate and acetylcholine potentials after 10 successive nerve volleys. The same experiment as in Fig. 1.
Ordinate: Amplitudes of end-plate potentials (○) and of acetylcholine potentials (●) as percentage of those of unconditioned end-plate potentials and of unconditioned acetylcholine potentials respectively. Each point and vertical line represent mean value and ± *S.D.* respectively calculated from five determinations. Abscissa: Intervals between the end-plate or the acetylcholine potentials and the conditioning stimuli

The amplitude of end-plate potentials and that of acetylcholine potentials at increasing intervals after conditioning stimuli are given in Fig. 2 as a percentage of amplitudes of end-plate and acetylcholine potentials which are not preceded by repetitive stimulation.

Essentially the same result was obtained from the preparation in which the activity of cholinesterase was completely inhibited by prostigmin 2×10^{-6}.

It is concluded from this experiment that the sensitivity of the end-plate to acetylcholine remains entirely unchanged even when the amplitude of the end-plate potential is markedly decreased after repetitive stimulation. This conclusion is in good agreement with the earlier suggestion[1,2] of the presynaptic nature of neuromuscular depression, but makes a sharp contrast with the result obtained by Thesleff in the rat. The difference between Thesleff's result and ours might be due to use of a different species; or, the diminution of the response to acetylcholine after conditioning stimuli observed in the rat might have been caused by the mechanical movements, since even a very minute movement suffices to abolish completely the response to acetylcholine.

We wish to thank Prof. H. Kumagai and Prof. S. Ebashi for their encouragement and advice.

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Actions of the Decarboxylase Inhibitor, α-Methyl-3,4-dihydroxyphenylalanine, in the Dog

Alpha-methyl-3,4-dihydroxy-DL-phenylalanine was shown by Sourkes[1] to be a potent inhibitor of the decarboxylation of 3,4-dihydroxyphenylalanine *in vitro*. This action was confirmed by animal studies in which the pharmacological actions of 3,4-dihydroxyphenylalanine and 5-hydroxytryptophan were decreased by administration of this compound[2,3]. Further evidence of the activity of α-methyl-3,4-dihydroxy-DL-phenylalanine *in vivo* was obtained by Smith[4], who reported that levels of serotonin in the brains of mice were decreased after parenteral administration of this compound. More recently, Oates *et al.*[5], in this Laboratory, observed a decrease in the formation of serotonin, tryptamine and tyramine from the precursor amino-acids when 1·0–6·0 gm./day of α-methyl-3,4-dihydroxy-DL-phenylalanine were administered to hypertensive patients. Associated with this decrease in amine production was the development of transient sedation and a lowering of the blood pressure.

In the present investigation, intravenous administration of α-methyl-3,4-dihydroxy-DL-phenylalanine was found to decrease the blood pressure and to produce sedation of the unanæsthetized dog, thus making it possible to study these actions of the inhibitor by pharmacological and biochemical techniques not

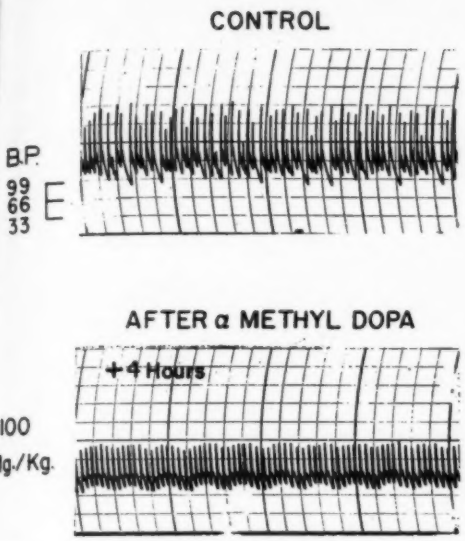

Fig. 1. Femoral arterial pressure of an unanæsthetized dog before (above) and 4 hr. after intravenous administration of 100 mgm./kgm. of α-methyl-3,4-dihydroxy-DL-phenylalanine. The scale to the left of the upper tracing is in mm. mercury. The heavy vertical lines signify 10-sec. intervals

applicable in man. Blood pressure was recorded from the femoral artery in five unanæsthetized, semi-trained, mongrel dogs by direct puncture or by cannulation of the artery under local anæsthesia. The pressures were measured electronically by means of a Statham transducer and Grass polygraph. In each animal, intravenous administration of 100–200 mgm./kgm. of α-methyl-3,4-dihydroxy-DL-phenylalanine produced reductions in arterial pressure ranging from 30/10 to 60/30 mm. mercury (Fig. 1). The hypotension did not begin until 2–3 hr. after drug administration and the maximum effect was usually not observed for 4–6 hr. The blood pressure returned to normal values within 24 hr., and in three of the animals repeated administrations of α-methyl-3,4-dihydroxy-DL-phenylalanine on 2–7 subsequent days produced equivalent hypotensive effects. At the time the blood pressure was reduced, there was no consistent change in heart-rate, no alteration in the response to tilting the animal through 90°, and no relaxation of the nictitating membrane. In most experiments, the animals appeared to be quietened by the inhibitor, but they were easily startled by noise. Injections of saline in the same animals had no effect on blood pressure recorded under identical conditions.

Heart contractile force was measured by a Walton strain gauge arch in seven vagotomized, anæsthetized, open-chest dogs as previously described[6]. The pronounced and prolonged increments in heart contractile force produced by 10–20 mgm./kgm. of L-3,4-dihydroxy-DL-phenylalanine or DL-metatyrosine, injected intravenously, were found to be completely inhibited by intravenous administration of 100–200 mgm./kgm. of α-methyl-3,4-dihydroxy-DL-phenylalanine. This total inhibition remained for 30 min. after drug administration and then gradually decreased so that the responses of 3,4-dihydroxy-DL-phenylalanine and metatyrosine were essentially back to control-levels in 4–6 hr. In these and similar experiments, the cardiovascular effects of l-norepinephrine, dopamine and tyramine were not significantly altered. The increments in heart-rate and contractile force produced by stimulation of the presynaptic fibres to the stellate ganglion were also not blocked by administration of α-methyl-3,4-dihydroxy-DL-phenylalanine, indicating that the function of the sympathetic ganglion and nerves was intact.

In order to correlate the pharmacological actions of the inhibitor with biochemical changes, preliminary determinations of decarboxylase inhibition and serotonin-levels in the brain (caudate nucleus) and norepinephrine in the heart (atria) were obtained in thirteen additional dogs. Eight dogs were killed by bleeding under barbiturate anæsthesia at 3, 5, and 24 hr. after intravenous administration of α-methyl-3,4-dihydroxy-DL-phenylalanine as shown in Table 1. Five dogs were killed in a similar manner to serve as controls. Serotonin-levels and decarboxylase inhibition were determined by previously described techniques[7]. Trichloracetic acid extracts of tissues were purified by absorption on alumina and the eluates were assayed fluorimetrically for norepinephrine by a modification of the trihydroxyindole method[8]. The high fluorescence of α-methyl-3,4-dihydroxy-DL-phenylalanine or of its metabolites remaining in the tissues prevented determination of norepinephrine levels in the 3- and 5-hr. samples. Accordingly, two additional experiments were carried out with a related decarboxylase inhibitor, α-methyl-3-hydroxy-DL-phenylalanine (α-methyl metatyrosine), which does not interfere with norepinephrine determinations.

Table 1. Effects of Decarboxylase Inhibitors on Levels of Serotonin in the Brain and Norepinephrine in the Heart

No. of dogs	Inhibitor	Dose (mgm./kgm.)	Time (hr.)‡	Serotonin (caudate nuclei) (μgm./gm.)	Norepinephrine (atria) (μgm./gm.)
5	Control	—	—	1·03	2·41
2	α-methyl dopa*	200	3	0·43	—
2	α-methyl dopa	200	5	0·78	—
2	α-methyl dopa	200	24	1·13	1·88
1	α-methyl dopa	500	24	1·00	1·77
1	α-M.M.T.†	200	5	0·41	1·74
1	α-M.M.T.	400	5	0·50	1·53

* α-methyl-3,4-dihydroxy-DL-phenylalanine.
† α-methyl-3-hydroxy-DL-phenylalanine.
‡ Time in hours after administration of inhibitors.

The results of the serotonin and norepinephrine determinations are shown in Table 1. As may be noted, there was a decrease in the serotonin-levels in the brain to values 60 per cent lower than those of the control animals in 3 hr., but in the 5-hr. specimens the serotonin-levels had returned to 80 per cent of control values. These changes in serotonin content appeared to be related to decarboxylase inhibition for in 3 hr. there was 40 per cent inhibition, but there was no inhibition demonstrable in the 5-hr. specimens. These *in vitro* determinations of decarboxylase inhibition, therefore, correlated well with the inhibition of the cardiac actions of 3,4-dihydroxy-DL-phenylalanine and metatyrosine *in vivo*. Norepinephrine-levels were decreased slightly 5 hr. after α-methyl-3-hydroxy-DL-phenylalanine and 24 hr. after α-methyl-3,4-dihydroxy-DL-phenylalanine. Serotonin-levels and decarboxylase inhibition were approximately the same in the specimens obtained 5 hr. after administration of α-methyl-3-hydroxy-DL-phenylalanine as in the specimens obtained 3 hr. after α-methyl-3,4-dihydroxy-DL-phenylalanine.

The results of this work indicate that the pharmacological effects of α-methyl-3,4-dihydroxy-DL-

phenylalanine in the dog do not appear to be related to decreased function of the peripheral sympathetic nerves or to an adrenergic blocking action. The preliminary biochemical work demonstrates that α-methyl-3,4-dihydroxy-DL-phenylalanine is a potent decarboxylase inhibitor and decreases serotonin-levels in the dog. These changes, however, did not appear to be temporally related to the pharmacological effects in the unanæsthetized dog. The decrements in norepinephrine-levels in the heart were not pronounced and were probably of little pharmacological significance since the response of the heart to stimulation of cardiac sympathetic nerves was unaltered. Additional work is being carried out to determine whether the pharmacological actions of α-methyl-3,4-dihydroxy-DL-phenylalanine and similar compounds are related to decarboxylase inhibition.

We wish to thank Merck, Sharp and Dohme for the supplies of α-methyl-3,4-dihydroxy-DL-phenylalanine and α-methyl-3-hydroxy-DL-phenylalanine.

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HÆMATOLOGY

Glucose-6-phosphate Dehydrogenase Deficiency, Haptoglobin Groups, Blood Groups and Sickle Cell Trait in the Bantus of West Belgian Congo

Since the demonstration that red blood cells of almost 10 per cent of American Negroes are deficient in glucose-6-phosphate dehydrogenase[1,2], one can suppose that the same defect exists in West Africa. The semi-quantitative test described by Motulsky[3] is particularly suitable for a rapid and efficient screening of the male subjects in a given population.

We made such a survey among the adult male Bantus of Leopoldville, capital of the Belgian Congo. More than 75 per cent of the inhabitants of this town originated from the west parts of the country. Among 522 adult males, we found 112 deficient subjects (21·5 per cent). This percentage is one of the highest hitherto discovered in adult males (Table 1). We have found by our own experience that, among deficient males, the glutathione stability test according to Beutler[4] gives the same results as the screening test proposed by Motulsky.

Table 1. Racial Incidence of Glucose-6-phosphate Dehydrogenase Deficiency in Male Subjects

Origin	Deficient percentage	Screening method
Sardinians (ref. 5)	8	Glutathione stability test
American Negroes (ref. 1)	8·8	Glutathione stability test
Iranians (ref. 6)	9·8	Glutathione stability test
Non-Ashkenazic Jewish (ref. 7)	11·2	Glutathione stability test
Nigerians (ref. 8) (two tribes)	6·14–16·9	According to Motulsky
Congolese Bantus	21·5	According to Motulsky

To obtain a more complete view of the genetic pattern of these Bantus, we performed a survey, determining in each of a group of 150 adult male subjects the following genetically inherited characteristics of the blood: A, B, O and D blood groups, sickle cell trait (paper and starch-gel electrophoresis at *p*H 8·6 coupled with falciformation test), haptoglobin groups (according to Laurell[9] on starch-gel electrophoresis) and glucose-6-phosphate dehydrogenase activity in erythrocytes (according to Motulsky).

Table 2

Origin	Phenotype percentage A	B	AB	O	Origin	Phenotype percentage D	d	D*
Congolese male Bantus	26·2	21·2	5·8	46·8	Idem	94	3·3	2·6
Belgians (Hubinont, ref. 10)	43·3	7·9	3·4	45·4	Belgians (Moureau, ref. 11)	82·4	17·6	

As already known, the phenotypes B and D are more frequent among Bantus than among Caucasians (Table 2). Nevertheless, the genes of A, B, O, and rhesus blood groups are not closely linked with the genes responsible for the glucose-6-phosphate dehydrogenase deficiency, as indicated by a χ^2 test.

Phenotype number	AB	A	B	O
Non-deficient	7	30	28	58
Deficient	2	11	5	15

$\chi^2 = 1·5$, P between 0·7 and 0·5.

The Congolese Bantus have one of the highest sickle cell trait incidence in the world. Figures of 25–30 per cent in the west and central parts of Belgian Congo are quoted by Vandepitte[12]. In our group of 151 male adult Bantus, we found 33 subjects with the genotype AS (21·8 per cent). Although almost equal in incidence, the enzyme deficiency and sickle cell trait do not appear to be linked.

Tested number	AS	AA
Non-deficient	25	93
Deficient	8	25

$\chi^2 = 0·376$, P between 0·7 and 0·5.

We did not find any person belonging to the haptoglobin genotype 0–0, as described by Allison[13]. It appears from the literature that the percentage of haptoglobin groups varies according to different races. Congolese Bantus present the highest percentage for haptoglobin group 1–1 and the lowest for haptoglobin 2–2 hitherto described (Table 3). There is no apparent linkage between the genes of glucose-6-phosphate dehydrogenase deficiency and those of haptoglobin groups.

Phenotype number	1–1	1–2	2–2
Non-deficient	62	42	5
Deficient	20	11	0

$\chi^2 = 1·72$, P between 0·3 and 0·5.

Table 3. Racial Incidence of Haptoglobin Groups

Origin	Phenotype percentage 1—1	1—2	2—2	Gene percentage 1	2
Indians (ref. 14)	1·8	14·6	81·7	0·09	0·91
Bushmen (ref. 15)	10·8	36	53	0·29	0·71
Swedish (ref. 16)	15	47	38	0·38	0·61
Ivory Coast (ref. 17)	49	42	9	0·70	0·30
Congolese Bantus	59	37·5	3·5	0·77	0·22

Summarizing, we can assert that Congolese Bantus are characterized by a high incidence of S hæmoglobin, glucose-6-phosphate dehydrogenase deficiency, haptoglobin 1, B and D blood group genes. These facts support the hypothesis of poor admixture of foreign blood in this population.

This particular genetic pattern induces some pathological consequences and raises a few questions.

(1) In the Bantu, the incidence of hæmolytic disease of the newborn by anti-D iso-antibodies is quite uncommon.

(2) Clinicians must bear in mind the possibility of hæmolytic crisis in patients lacking glucose-6-phosphate dehydrogenase, induced by drugs like 8-aminoquinolines, naphthol derivates, sulphones and nitrofurazone. This last-named drug is certainly dangerous as demonstrated by one of us in a Bantu patient[18]. As the enzyme activity in erythrocytes of deficient Bantus is 1/5–1/8 of a normal control (Sonnet, J., and Haumont, A., unpublished work), it is likely that deficient Bantus are not so sensitive as Caucasian males, whose enzyme deficiency is almost complete.

(3) The parallelism between the high incidence of sickle cell trait and enzymatic deficiency in the Bantus, as compared with American Negroes, suggests that, in a tropical environment, an identical underlying cause could exert a positive selection on both kind of carriers. As suggested by Motulsky, the mechanism of this advantage could be a protection against *Plasmodium falciparum* infection.

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Hæmagglutination of Cells treated with Antibiotics

Previously, we directed attention to the occurrence in certain sera of an antibody-like factor capable of causing hæmagglutination of group O, Rh-negative red cells modified by exposure to penicillin[1,2]. The reaction appears to be specific and is inhibited by preliminary mixing of the sera with penicillin.

We have extended this work to try to determine the presence of hæmagglutinating factors for erythrocytes modified by other antibiotics. Negative results were obtained with streptomycin, dihydrostreptomycin and tetracycline. However, from a series of 1,700 routine hospital blood bank sera tested, six were found capable of agglutinating chloramphenicol-modified cells. Titres ranged from 1/2 to 1/128, when the sera were diluted in 0·85 per cent saline and incubated at 37·0° C. for 1½ hr. followed by spinning at 1,000 rev./min. for 1 min. before reading.

Sensitized cells were prepared by adding 250 mgm. of chloramphenicol to 4·0 ml. of fresh blood in 4·0 ml. of Alsevers solution. Incubation at 37·0° C. for 1 hr. was followed by storage at 4·0° C. overnight. For use, 2·0 ml. of suspension were washed four times in 20·0 ml. of saline and resuspended to make an approximate 10 per cent suspension. Red cells exposed to a mixture of penicillin and chloramphenicol were agglutinated in the presence of each homologous positive serum.

The hæmagglutinin against chloramphenicol-modified cells appears to be similar to that against penicillin-modified cells. Both are stable on heating, and the reaction is inhibited in the presence of the appropriate antibiotic. In each instance dilution in 30 per cent bovine albumin solution leads to an approximate four-fold reduction in titre. None of the sera against chloramphenicol-modified cells gave a positive antiglobulin test. Solutions of urea (20 per cent), sodium salicylate (5·0 per cent), and glycerol in saline (20 per cent) inhibited both reactions, suggesting that the nature of the physical associative interaction between the molecules may be one of hydrogen bonding.

We considered previously the possibility of an immune origin for the penicillin-modified cell hæmagglutinin, penicillin acting as a haptene in conjunction with some tissue protein[1]. Of 30 such sera 28 were derived from patients with a history of previous penicillin therapy. However, of the six sera agglutinating chloramphenicol-modified cells, five were obtained from patients with no previous history of chloramphenicol treatment. The highest titre serum (1/128) came from a patient aged six years who had apparently never received any antibiotic therapy. It may be that there has been previous antigenic stimulation by some substance or compound with a common determinant group to chloramphenicol or, alternatively, the reaction might be due to the presence of some naturally occurring factor in certain sera. If so, the specificity of the reaction seems remarkable. One serum will not agglutinate erythrocytes modified by the heterologous antibiotic, nor will penicillin inhibit the agglutination of chloramphenicol-treated cells with the homologous serum and vice versa. If not of immune origin, the apparent narrow specificity of the reaction requires further elucidation.

By starch-gel electrophoresis we have demonstrated hæmagglutinating activity in the pre-albumin moiety of positive sera against penicillin-modified cells. In

only one of 24 sera have we found activity in the γ-globulin fraction, and this serum gave a positive antiglobulin test in addition to having the characteristic agglutinating fraction in the pre-albumin zone. The suggestion is made that some antibiotics may function as haptenes and produce true immune hæmagglutinin of γ-globulin type, but that certain sera may contain an additional fraction in the pre-albumin moiety which may or may not be of immune origin. The nature and further characteristics of this factor are under investigation.

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Separation of Human Serum Albumins

The presence of several protein components in human serum albumin was recently demonstrated[1-3] by two-dimensional zone electrophoresis (filter paper followed by starch gel)[4]. Their identity with albumin was determined by two-dimensional zone electrophoresis[3]. The proteins are not related to those described by Knedel[4] or by Gitlin *et al.*[5]. During our work on normal human sera, several new proteins were observed which had the same mobility as albumin by filter paper electrophoresis (at *p*H 8·6), but which when subjected to electrophoresis in starch gel in a discontinuous system of buffers[7] migrated more slowly than albumin. These proteins can be divided into two main groups: the members of the first group are immunologically related to albumin and one of them is present in sera in about 50 per cent of all normal adults and children. These proteins will be referred to as 'albumin-like proteins'.

A second group of proteins was also found which possessed a mobility on filter paper similar to, but not identical with, that of albumin. They migrate at the same rate as the 'slow' portion of the albumin and in starch gel can be separated from albumin only in a discontinuous system of buffers. These proteins are not immunologically related to albumin and will be referred to as 'quasi-albumins'. Their presence in sera was found in 61 of the 64 individuals screened.

Proteins of either or both these groups may be present or absent in a serum for a given individual. Fig. 1, *a*, *b*, *c*, shows diagrammatically the three possible combinations of the occurrence of these proteins. A detailed description of the distribution and some of the properties of the proteins will be given elsewhere.

This work was supported by the Michigan Kidney Disease Foundation.

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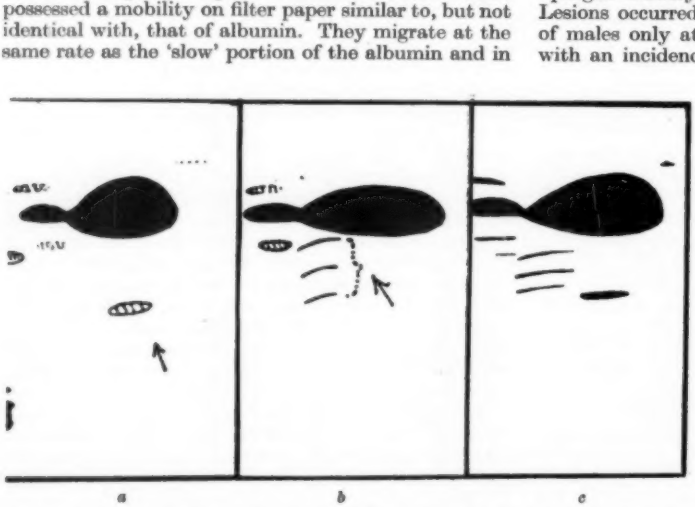

Fig. 1. Diagrams (traced from photographs) of two-dimensional zone electrophoretic patterns of human sera showing the separation of albumins (only albumin(s) and α-1-globulin regions shown). *a*, position of the 'albumin-like' protein; *b*, positions of 'quasi-albumins'; *c*, pattern of an individual with both groups of proteins

PATHOLOGY

Spontaneous Cytomegalic Inclusion Body Disease involving Lacrimal Glands of Cæsarian-derived (so-called) Pathogen-free Rats

Histological study of lacrimal glands in laboratory rodents revealed cytological changes, for example, nuclear polymorphism and inclusions characteristic of cytomegalic inclusion body disease in rats of the Sprague–Dawley strain; no other organs were involved. Lesions occurred in both intra- and ex-orbital glands of males only at an age of six months or older and with an incidence of 100 per cent. Similar observations have recently been published for eight rat colonies of different geographical locations[1]. The disease is apparently widespread among laboratory rats. Guinea pigs, a variety of mouse strains, both golden and Chinese hamsters, Wistar and Fisher rats, either commercially obtained or bred and raised in the Roscoe B. Jackson Laboratory, were free of the disease.

Our Sprague–Dawley colony was derived from stock animals purchased several years ago from a local breeding farm. For a number of years now, this breeder has since specialized in the production of Cæsarian-derived pathogen-free animals. Routinely Cæsarian-derived Sprague–Dawley pups are foster-suckled on germ-free Lobund strain mothers (alpha-colony) and on attaining breeder size

transferred to pathogen-free surroundings. Cæsarian sections are performed in sterile plastic isolaters ('Trexler' type) and pathogen-free conditions for Cæsarian-derived Sprague–Dawley breeders maintained by an elaborate barrier system (sterilizer and lock system). Eight generations of pathogen-free Cæsarian-derived Sprague–Dawley rats have so far been obtained, interrupted only by introduction of new germ-free Lobund rats.

Pathogenic bacteria and parasites have never been demonstrated from any of the Cæsarian-derived Sprague–Dawley rats. Lacrimal glands, however, from *both* males and females (100 per cent) disclosed cytomegalic inclusion body disease at about six months or older. Transmission experiments were conducted with cell-free filtrates prepared by grinding glands with pestle and mortar, 'Carborundum' and tissue culture fluid (dilution 1:10) fortified with aqueous penicillin-streptomycin, centrifuged at 3,000 r.p.m. for 30 min. and filtered through bacteria-tight *EK*-Seitz filter pads. Sprague–Dawley new-borns were inoculated intraperitoneally, 0·1 ml., and intracerebrally, 0·03 ml. Death of 20 per cent occurred within one month from systemic cytomegalic inclusion body disease. Surviving animals, both males and females, killed at intervals of 55, 63 and 71 days, showed extensive lacrimal gland involvement only. Similarly inoculated new-born mice remained healthy and no microscopic lesions were detected.

The problem of viruses in both pathogen- and germ-free animals should be investigated systematically and adequate tests devised for virus detection. Although it is possible to raise animals in the absence of bacteria, protozoa and helminths, elimination of all viruses may be more difficult. It seems significant that at the Lobund Institute filtrable agents had been isolated from cæca of rats that were raised germ-free for several generations[2]. Tentative identification of these agents classified them serologically in the group of *ECHO* viruses. Also, at the National Institutes of Health, from the salivary glands of germ-free rats an agent was isolated that could be propagated on rabbit kidney cells[3].

From evidence cited it would seem that the pathogen- and germ-free state does not necessarily apply to viruses. As shown here, so-called pathogen-free animals may in fact contain infective viruses. Although viruses may be occult both clinically and microscopically, viral antibodies may or may not be detectable in infected host's serum. The presence or absence of humoral antibodies to cytomegalic inclusion body disease virus in so-called pathogen-free Sprague–Dawley rats is under investigation; also, attempts are being made to find out the virus state prevailing in this disease as compared with latent murine lymphocytic choriomeningitis and swine influenza in which neither virus nor viral antibodies are detectable by direct means. Transmission of the virus in pathogen-free rats has to be studied from an epidemiological viewpoint; it may enter postnatally (for example, viruria, occular discharge) or prenatally by way of placental transfer (following viræmia, for example, herpes). In pathogen-free rats virus multiplication does indeed occur in lacrimal glands of females, but the presence of virus in conventional females appears to be masked. Morphological studies with the light- and electron-microscope will be presented in a separate description of the fine structure, as well as pathogenesis of inclusions.

This investigation was performed in part during tenure of appointments at the Children's Cancer Research Foundation and the Departments of Pathology, The Children's Medical Center and Harvard Medical School, Boston, Massachusetts.

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Schistosomiasis and Urinary β-Glucuronidase Activity

Boyland, Wallace and Williams[1] have shown that the activity of urinary β-glucuronidase is increased in patients suffering from bladder cancer and suggested that 'spontaneous' urinary bladder cancer could be caused by unmasking carcinogens which were inactive as β-glucuronides, but active when liberated by β-glucuronidase in the urine.

Bladder cancer is not uncommon in East Africa. The Cancer Registry of the Kampala Cancer Research Committee has noted 68 cases out of a total of 1,800 cancer cases over the past 4 years.

The role of schistosomiasis in the ætiology of bladder cancer is controversial[2], and as part of a programme of investigations into the background of cancer in East Africa, the effects of schistosomiasis on urinary β-glucuronidase activity are being studied. 24-hr. samples of urine are collected and the β-glucuronidase activity is estimated by a slight modification of the method of Boyland, Gasson and Williams[3].

Preliminary results on a limited number of male East African natives showed that the normal range of activity was fairly narrow and was lower than that quoted by Lewis and Plaice[4] for Europeans on a units per ml. basis, but slightly higher when expressed as activity per 24 hr.

The urine of patients suffering from intestinal schistosomiasis (*Schistosoma mansoni*) showed an increase in activity above the normal range and those with urinary schistosomiasis (*S. hæmatobium*) showed a well-marked increase.

Table 1. URINARY β-GLUCURONIDASE ACTIVITY

Group	N	Units/ml.	*S.D.*	Units/24 hr.	*S.D.*
African normals	18	0·95	0·29	1,475	120
S. mansoni	8	1·59	0·91	1,970	573
S. hæmatobium	7	3·08	2·18	4,560	1,710
European normals[4]	32	1·05	0·55	1,405	893

After treatment with 'Nilodin' (lucanthone hydrochloride, Burroughs Wellcome) or sodium antimonyl tartrate, the activity of the enzyme dropped rapidly and, in cases where the series were long enough, reached the normal range, and in one case was still normal after 58 days. This drop was well marked in the *S. hæmatobium* series, but longer series are required for *S. mansoni*. Details of the longest series are given in Table 2.

Since urinary schistosomiasis is accompanied by hæmaturia, two cases of sterile hæmaturia unassociated with schistosomiasis were studied. Both gave results which were within the normal range. Exten-

Table 2. The Effect of Treatment on β-glucuronidase Activity

Schistostoma hæmatobium						*Schistostoma mansoni*					
Case	Pre-treatment		Post-treatment			Case	Pre-treatment		Post-treatment		
	units/ml.	units/24 hr.	Day	units/ml.	units/24 hr.		units/ml.	units/24 hr.	Day	units/ml.	units/24 hr.
OB	6·00	7,020	1	4·96	6,150	*OL*	1·25	2,920	1	0·96	1,330
			2	3·52	4,720				2	0·90	1,190
			3	2·30	3,400						
OP	6·57	7,750	1	4·95	5,000	*NA*	3·66	2,120	1	2·93	1,640
									2	2·80	1,490
BA	1·20	3,480	1	0·97	2,570	*OK*	1·74	3,000	1	1·74	2,460
			8	0·64	1,760						
MW	2·50	5,580	1	2·10	5,100	*OC*	—	—	1	1·06	2,440
			8	1·23	1,970				2	1·54	3,250
									3	1·36	2,900
KA	2·63	4,400	1	1·85	3,090	*AL*	1·75	1,980	6	0·93	1,020
			2	2·63	2,820						
			3	2·58	1,680						
			58	1·32	1,630						

sions of this work are being undertaken and will be published elsewhere.

I am indebted to Prof. J. N. P. Davies of the Department of Pathology, Mulago Medical School, Makerere College, for his support and interest and to Dr. Vaizey and Dr. Rosanelli of the Mulago Hospital for their kind co-operation.

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Induction of Tumours by Polyoma in Adult Hamsters

The relative non-susceptibility of adult versus new-born or very young animals to tumour induction by certain viruses has been interpreted as the expression of either of two possibilities: (1) presence in the young animal of cells intrinsically more susceptible to the virus infection and to the proliferative response; (2) lack in the young animal of an adequate immunological response.

In the case of tumour induction by polyoma, much emphasis has been placed on the fact that it is the undifferentiated mesenchymal cells in the new-born hamster which are susceptible to the proliferative response[1]. Thus the proliferative response to infection and the appearance of the tumour would depend on the availability of such cells. Results presented here show that the tumour can be induced by polyoma in adult hamsters, and special attention has been paid to the influence of the immunological process on the outcome of polyoma infection in terms of tumour production. Twenty male Lakeview hamsters 6 weeks old were divided into two groups: 10 animals were inoculated subcutaneously and 10 intra-peritoneally with a single dose of 0·2 mm. of a tissue culture preparation of polyoma virus (*M*–11–9*A*). Of the group inoculated intraperitoneally, 5 died between the 8th and 15th week after inoculation; only one could be autopsied, and the cause of death was found to be a massive intraperitoneal hæmorrhage, originating in a broken hæmorrhagic cyst in the liver; a similar picture was found in another animal dying 24 weeks after inoculation. In this same group, one animal, 13 months after receiving the inoculum, developed a subcutaneous nodule in the left flank which rapidly enlarged, reaching a diameter of 5 × 3·5 × 5 cm. In no other areas were proliferation or necrotizing lesions observed. In the group inoculated subcutaneously, 3 animals were lost; 4 developed subcutaneous tumours 24, 35, 36, and 44 weeks after the inoculation, respectively. The tumours were very similar to those observed after inoculation in new-born hamsters, being characterized by large, hæmorrhagic necrosis with very pronounced vascular formations in the viable part. The remaining 4 animals in the two groups were killed 14 months after inoculation and failed to show proliferative lesions.

In summary, 7 out of 11 hamsters which were inoculated at 6 weeks of age and in which necropsy could be done showed tumours of the liver or of the subcutaneous tissue 3–14 months after inoculation. No other tumours were found in any other organ, suggesting that the tumour had developed at the site of inoculation. The tumours were in all respects identical to those observed in new-born animals, and, in the case of the subcutaneous lesions, grew very rapidly. Hæmagglutinin-inhibiting antibodies were found in all 7 of the animals tested, 3 with tumours and 4 without tumours at 11 and 14 months after the virus inoculation. Thus the only difference between new-born and adults after an equal amount of virus inoculation appears to be that in adults the latent period preceding the appearance of the tumour is greatly prolonged and that the tumour appears only at the site of inoculation. Cells capable of proliferative and neoplastic response to polyoma must, then, be present and available to the virus in the adult animal as well—probably throughout its life—although it cannot be excluded that the number and the areas of these cells may be decreased in the adult animal. It would seem that the difference between the new-born and adult animals can be better explained on the basis of immunological activity. Several observations point in this direction: high titres of polyoma hæmagglutination-inhibiting antibodies are reached in the adult hamsters by the 4th–5th day, while in the new-born similar titres are found only at the 6th–7th day (Defendi, V., unpublished work); inoculation of adult lymphoid tissue in the new-born

before the virus is given increases the latency period before tumour appearance and decreases the actual number of tumours per animal[3]; when the lymphoid tissue inoculated originated from animals with antibodies to polyoma, the actual incidence of tumours decreases by 60 per cent, with tumours present only at the site of inoculation (Defendi, V., unpublished work). Briefly, when inoculated with adult lymphoid tissue, the new-born hamster responds to the polyoma in much the same way as the adult. One attractive hypothesis that could be formulated is that in the adult animal the considerable replication of the virus found in the new-born[3] does not occur, or that the virus is rapidly neutralized, decreasing the probability of tumour initiation in organs remote from the site of inoculation. It should also be kept in mind that the virus concentration at equal inocula is much lower in the large adult animal than in the new-born.

In relation to the problem of new-born versus adult susceptibility to polyoma, it has been found in another animal (mouse) that adult salivary gland *in vitro* is just as susceptible to tumour induction by polyoma as is the fœtal gland, and that X-ray treatment makes the adult mouse susceptible to the oncogenic action of polyoma[4].

This work was supported in part by United States Public Health Service Grant *C*–4534 (National Cancer Institute) and by Grants *E*–88 and *E*–89 from the American Cancer Society.

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HISTOLOGY

Mineralization of Rat Enamel

The initial stages of the mineralization of tooth enamel have been studied using electron microscopical techniques applied to ultra-thin sections of developing molars and incisors of 1–10-day-old white rats (*Rattus norvegicus*). The specimens were fixed in either Palade's[1] or Dalton's[2] fixative, embedded in 'Araldite' and cut without demineralization using a diamond knife.

Tooth enamel is a highly mineralized tissue consisting of tightly packed orientated apatite crystals in intimate association with an organic substance 75 per cent of which contains citric acid, peptides and a glycoprotein, the remainder being a protein of the keratin type. There is considerably more mineral than organic matter, the mineral contributing approximately 96 per cent by weight, organic material approximately 1·5 per cent and the rest water.

It is generally accepted that the keratinous organic matrix of the enamel is fibrous and that its formation precedes the mineralization process. The exact role of the ameloblast cell in enamel formation is a matter still under consideration. I believe[3] that fibrillogenesis occurs extra-cellularly, the fibres forming from a granular precursor substance which is elaborated and secreted by the ameloblasts, the

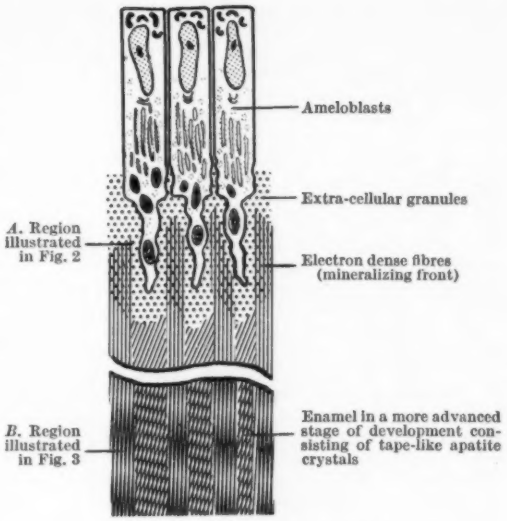
Ameloblasts
Extra-cellular granules
A. Region illustrated in Fig. 2
Electron dense fibres (mineralizing front)
B. Region illustrated in Fig. 3
Enamel in a more advanced stage of development consisting of tape-like apatite crystals

Fig. 1. Diagram of amelogenesis

granules being discharged from the formative end of the cell into the extra-cellular environment. This concept is illustrated diagrammatically in Fig. 1. The newly secreted organic material in the extra-cellular region between the cells appears first to consist of granules with an electron density equal to that of the intra-cellular 'Palade' granules. Some granules then become arranged in poorly defined columns giving rise to what appear to be orientated fibres. An abrupt change to very electron dense fibres marks the next stage in enamel formation (Fig. 2). In an earlier publication[4] I designated the region undergoing this change as the 'formative front' of the enamel matrix. This 'front' is marked by a striking contrast between the granules (diam. 50–70 Å.) which have a low electron scattering capacity and fibres (ε0–100 Å. × 0·1μ–1·0μ) (Fig. 2) which have a much greater electron-scattering property. The fibres, which have a periodicity of 50–70 Å., become arranged in parallel rows, the strands of which appear to be cross-linked in places by electron-dense granules; this results in an appearance rather like the rungs of a ladder. In the depths of the enamel where mineralization is more advanced, both granules and fibres are replaced by 'tape-like' crystals which give a diffraction pattern characteristic of apatite. The transition from fibres to 'tape-like' crystals, which might be expected to be a gradual process, is difficult to detect with certainty in 'bright-field' electron micrographs.

Diffraction patterns characteristic of apatite were obtained from selected areas of the formative front of the enamel matrix and from various places in the deeper layers of the newly formed enamel up to 30μ from the formative zone (positions *A* and *B*, Fig. 1). A progressive change from extremely small crystallites of approximately 100 × 500 Å., to larger crystallites of approximately 500 × 1000 Å., was calculated from the angular breadth of the diffracted beam from the 002 and 300 crystal planes. The smallest crystallites are situated in the electron dense region of the formative front of the enamel matrix (Fig. 2) whereas the larger crystallites are situated away from the

formative zone in the deeper layers of the enamel matrix.

Crystallite size calculated from the orientated electron diffraction patterns is greater than the observed size of the electron-dense particles (50–70 Å.) associated with the electron-dense fibres in transmission electron micrographs (Fig. 2). Calculation of crystallite size from the breadth of diffraction lines is most accurate when applied to diffraction patterns from cubic crystals where the crystallite size falls within the range 100–1000 Å.[5]. Cubic and non-cubic crystals below this size-range may therefore give diffraction patterns having poor intensity, and a diffuseness which contributes to large errors in calculations of crystallite size.

By interposing a small aperture below the object plane in such a way as to permit the passage of only part of the diffracted electron beam, it is possible to produce a 'dark-field' diffraction image which shows the position and size of those crystallites suitably orientated in respect to the electron beam. Such an image appears as a bright point on a dark background. Measurements of the crystallites visible in dark-field micrographs of the formative front reveal a size-range of 50–100 Å. in the zone nearest to the forming front. Moreover, the bright regions in dark-field correspond in position to the electron-dense regions in bright-field electron micrographs.

From the evidence provided by the diffraction patterns, and both dark-field and bright-field electron micrographs, it is concluded that the abrupt change to a high electron density of the fibres at the formative front is due to the multiple deposition of very small apatite crystallites which form centres from which

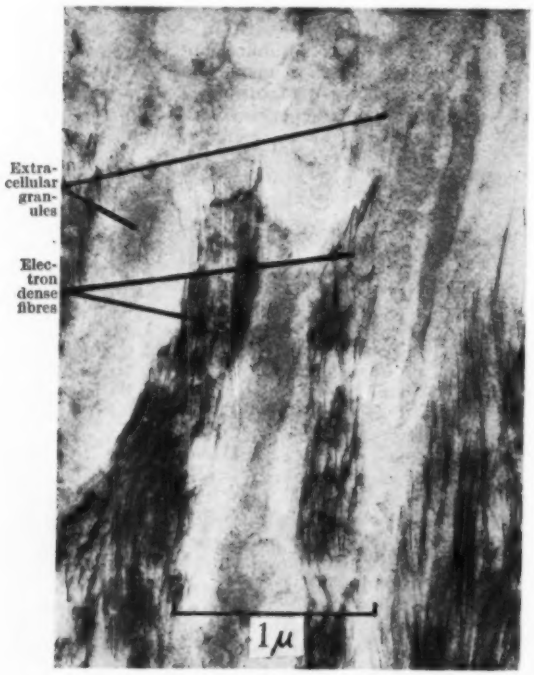

Fig. 2. 'Mineralizing front', the junction between extra-cellular granules and electron-dense fibres, region *A* in Fig. 1

Fig. 3. Tape-like crystals of enamel in a more advanced stage of mineralization, region *B* in Fig. 1

further crystal growth takes place. This growth leads to the formation of the 'tape-like' crystals which can be obtained from the deeper layers of the enamel (Fig. 3). As a result of these observations I would now prefer to call this region of changing electron density the 'mineralizing front' rather than the formative front of the enamel matrix.

It has been suggested by Frank and Sognnaes[6] that in enamel mineralization, crystal nucleation and growth are by epitaxy with the organic matrix. This may well be true since in dark-field electron micrographs small crystals can be detected spaced by regular intervals. These intervals may presumably represent the position of some structure which is not opaque to electrons and which could therefore represent the position of the organic material. Epitaxy could also explain the particularly high degree of orientation of the apatite crystals in different regions of tooth enamel. Crystallization by epitaxy commencing from multiple centres could also influence the mode of crystal growth and perhaps regulate size and packing of the crystals.

The work was assisted by grants from the Medical Research Council, and the Yarrow Research Fund of the London Hospital Medical College.

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RADIOBIOLOGY

Radioactivity in Wool

With the development of highly sensitive γ-ray detectors, it has become possible to carry out non-destructive measurements of the low levels of γ-ray activity emitted by various biological materials. Since γ-ray measurements may be made rapidly without any special preparation or modification of the material measured, the application of this technique to agricultural research problems has much to recommend it. The advantages of the non-destructive γ-ray measurement technique appear to justify exploratory research to determine if such measurements may be used to estimate other factors to which they may be related[1].

In the course of such a line of exploratory research γ-ray measurements were made on raw and scoured wool. A recently developed low-level γ-ray detector employing plastic scintillators was used in this work[2]. Simultaneous two-channel operation was used in making γ-ray radioactivity measurements. The upper channel was set to measure γ-radioactivity of from approximately 1·2 to 2·5 MeV. energy. The lower channel measurements represented γ-radioactivity of from approximately 0·6 to 1·0 MeV. energy plus a substantial spill-over of counts from the upper channel energy band. When a standard consisting of 1·00 lb. of potassium chloride mixed with 13·00 lb. of granulated sugar was counted using these channel settings, the net counting-rate for the potassium-40 γ-radioactivity in the lower channel was approximately 80 per cent of the net counting-rate observed in the upper channel. Since the resolution of our system is not adequate to identify unknown γ-ray emitters, no corrections for spill-over have been applied in Table 1.

Table 1. γ-Ray Radioactivity of Samples of Grease Wool and Scoured Wool

Samples	Lower channel (net counts/min./lb.)	Upper channel (net counts/min./lb.)
	Mean ± *S.D.*	Mean ± *S.D.*
Maryland grease wool	690 ± 111	731 ± 129
New Mexico grease wool	1,382 ± 57	669 ± 101
Idaho grease wool	1,565 ± 107	929 ± 76
Idaho scoured wool	46 ± 12	25 ± 4

γ-Ray measurements were made with samples of grease wool weighing between 8·6 and 10·0 lb. The grease wool samples were placed for counting in a fibre drum 11½ in. in diameter and 21 in. long. The wool had been sheared in the spring of 1959 from sheep from Maryland, New Mexico and Idaho. The 6 samples of Maryland grease wool were taken from pooled wool, while the 6 samples from the other locations were each taken from individual fleeces. Measurements were also made of 6 scoured wool samples from 1958 Idaho wool. Each of these samples, which weighed between 4·0 and 5·6 lb., was an entire fleece.

The mean values and standard deviations for each group of 6 samples are presented in Table 1. The scoured wool samples emitted a much lower amount of γ-ray radioactivity than did the grease wool samples. Domestic grease wool may contain 25–80 per cent impurities[3]. The major impurities in grease wool are grease, suint, and dirt. The suint or dried perspiration is quite high in potassium content. Since all potassium in Nature contains an essentially constant proportion of the γ-ray emitter, potassium-40 (ref. 4), with an energy of 1·46 MeV., it is probable that the upper channel measurements reflect the presence of suint in the grease wool samples. Grease wool may contain 5–40 per cent dirt content[5]. It will be noted that the lower channel net counts per min. per lb. values for grease wool from the three locations vary more widely than do the upper channel values. Natural radioactivity from soil[6] as well as fall-out debris may be factors contributing to the lower channel counts.

The γ-ray radioactivity of the clean wool is only a small fraction of that found in grease wool. Thus the bulk of the content of grease wool emitting γ-rays is associated with its impurities. Work is being carried out in our laboratories to determine if γ-ray measurements, which can be made non-destructively in a few minutes, may serve as an index for predicting the percentage of impurities present in grease wool and the relative proportions of the major impurities.

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An Effect of Tritiated Thymidine on the Incorporation of Thymidine into Chromosomal Deoxyribonucleic Acid

In the course of a series of experiments designed to test for possible anomalies in the synthesis and behaviour of chromosomal deoxyribonucleic acid resulting from the use of tritiated thymidine, the possibility arose that the tritiated precursor could interfere with the normal course of synthesis and partition by destroying the integrity of the deoxyribonucleic acid molecule or otherwise modifying the normally operative control mechanisms. This possibility was examined by comparing the incorporation of thymidine labelled with carbon-14 into meristematic cells in the presence and absence of tritiated thymidine.

Vicia faba roots were incubated for a period of 10 hr. in three different media: ^{14}C-2-thymidine (New England Nuclear Corporation), 2·7 μc./μmole, 1 μc./ml.; tritiated thymidine (Schwarz Bio Research Inc.), 3,000 μc./μmole, 5 μc./ml.; ^{14}C-2-thymidine and

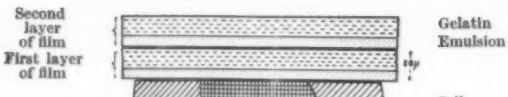

Fig. 1. Diagrammatic illustration of a two-layer stripping film preparation

tritiated thymidine with a final specific activity of 16 μc./μmole and a concentration of 6 μc./ml. The roots were fixed, briefly hydrolysed, squashed and covered with two layers of Kodak *AR*-10 stripping film (Fig. 1). After 19 days of exposure the autoradiographic preparations were developed and analysed by visual grain counts. The first film layer served as an effective screen against tritium β-particles: relative to the first layer, grain densities in the second layer for carbon-14 were about 50 per cent and for tritium about 0·5 per cent. The second layer over cells labelled with tritium alone showed a grain density over nuclei which was only 2 above background. This number was subtracted from the counts of doubly labelled nuclei in the main analysis which was carried out in the second layer of ^{14}C-thymidine and ^{14}C-thymidine-^{3}H-thymidine labelled cells. The results of this analysis are plotted in Fig. 2 and represent pure carbon-14 grain numbers.

These results demonstrate that tritiated thymidine enhances the incorporation of thymidine labelled with carbon-14 and lead to the conclusion that incorporation of tritiated thymidine into deoxyribonucleic acid cannot be assumed to measure normal amounts of synthesis of the acid but does in fact bring about modifications. (The possibility that carbon-14–thymidine itself produces similar difficulties is theoretically less likely, but cannot be easily evaluated in an experiment of this design.)

It is of interest to note in this connexion recent reports of chromosome breakage and reduction in mitotic index[1], increased mutation frequency[2] (both attributed to tritiated thymidine) and the demonstration of structural modifications in tritiated deoxyribonucleic acid[3]. These results, together with those presented here, form a broad spectrum of detectable modifications in the deoxyribonucleic acid molecule brought about by the introduction of tritium-labelled components. The finding of Pelc and LaCour[4] of excessive incorporation of tritiated thymidine in cells where similarly extensive incorporation has not been noted with other labelled precursors, and the apparent lack of quantitative correspondence between tritiated thymidine incorporation and cytochemically demonstrable deoxyribonucleic acid increase in puffed regions of polytene chromosomes[5,6] may also prove to be examples of tritiated thymidine effects rather than evidence for metabolic activity of deoxyribonucleic acid.

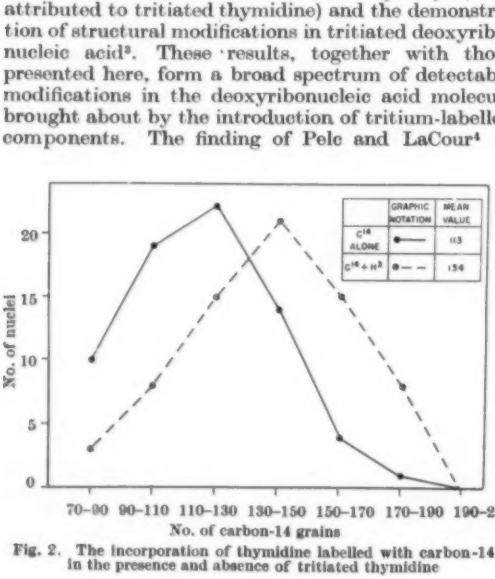

Fig. 2. The incorporation of thymidine labelled with carbon-14 in the presence and absence of tritiated thymidine

The results reported here are not unexpected in view of the extremely high ionization density produced by tritium β-particles and the complex structure of the deoxyribonucleic acid molecule[7,8]. It is somewhat surprising that a measurable effect is elicited by tritiated thymidine in as low a specific activity as was used in the present experiment (less than 16 μc./μmole). The explanation of this circumstance may lie in the sensitivity of the detection system we have employed, that is, the use of a tracer to measure disturbances produced by another.

A discussion of the possible nature of the modifications introduced by tritiated thymidine into chromosomal deoxyribonucleic acid behaviour is in preparation and will be published together with further data on the effect of this precursor on total levels of deoxyribonucleic acid.

This work was supported by a National Institutes of Health grant (*C*-3276) and funds from the Wisconsin Alumni Research Foundation.

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Influence of Thyroid on Radiophosphorus Metabolism in Fish

Information concerning the endocrinological regulation of mineral metabolism in fishes is lacking. The problem is particularly interesting because the parathyroid gland which regulates the normal calcium and phosphorus content within the body in higher vertebrates is supposed to be absent in fishes. I have tried to find out if the thyroid had any control over the mineral metabolism in yearling salmon, *Salmo salar* L.[1,2]. Since surgical thyroidectomy is not possible in fishes, an attempt was made to destroy the thyroid follicles by anti-thyroid drugs. Successful destruction could not be achieved since the fishes were treated with thiourea for a very short time. The result of the experiments suggested that when fish were treated with thiourea for a short time, thyroid was indirectly stimulated by thyrotropin secreted by the pituitary and so the uptake of phosphorus-32 was more than in the controls; in thyroxine-injected fish the uptake was a maximum because the stimulation was direct.

Complete thyroidectomy has been achieved by repeated injections of radioiodine[3]. In the present

investigations, goldfish, *Carassius auratus* L., were injected intraperitoneally with 40, 30, 20 and 10 μc. of iodine-131 respectively for 4 months—one injection every month. Histological examination of the thyroid after this period showed that radioiodine had selectively destroyed all the thyroid follicles. Another group of goldfish were injected with 0·4 ml. of 0·1 per cent thiourea three times a week for 2 weeks. Then for the next 3 weeks the volume of injection was reduced to 0·2 ml. The thyroid in this case became hypertrophied and hyperplastic, with increased number of very small follicles. The height of the cell was greatly increased and the colloid was very scanty. A third group of goldfish was maintained to serve as control. For every injection proper precautions were taken not to disturb the osmotic balance in the fish.

The foregoing groups of fishes were then subjected to three different sets of treatments. Fishes were given three injections of physiological saline, extract of acetone-dried goldfish pituitary gland (to supply them with additional quantity of thyrotropin) and 0·1 per cent L-thyroxine (Nutritional Biochemical Corp.) respectively on alternate days. The volume injected at a time was 0·2 ml. Radiophosphorus was then added to all the aquaria. The antibiotic tetracycline was put in the aquaria to eliminate any bacterial activity. Table 1 shows the amount of radiophosphorus absorbed in various sets.

Table 1. PHOSPHORUS-32 ABSORBED BY THE VARIOUS GROUPS OF FISHES INJECTED WITH PHYSIOLOGICAL SALINE, PITUITARY, AND THYROXINE

	Injections given		Phosphorus-32 absorbed (per cent)
Iodine-131-injected fish	*A*	Physiological saline	22·9
	B	Pituitary	24·6
	C	Thyroxine	34·6
Thiourea-injected fish	*A*	Physiological saline	26·7
	B	Pituitary	37·9
	C	Thyroxine	45·9
Controls	*A*	Physiological saline	45·0
	B	Pituitary	58·3
	C	Thyroxine	66·2

The conclusions drawn from the experiments are that radioiodine completely destroyed the thyroid follicles and so the radiophosphorus uptake was low (22·9 per cent), approximately half that of the controls (45·0 per cent). Injections of pituitary extract did not have much effect (uptake 24·6 per cent). This could be explained by the complete destruction of thyroid; thyrotropin of the pituitary which has the thyroid-stimulating function could not produce any effect. But the injections of the thyroid hormone, thyroxine, increased the uptake from 22·9 to 34·6 per cent (50 per cent increase).

The uptake of radiophosphorus in thiourea-injected fishes (26·7 per cent) resembles that of fishes injected with iodine-131, but the injections of pituitary extract increased the uptake in these fishes from 26·7 to 37·5 per cent (40 per cent increase). This is because the thyroid follicles had only become hypertrophied and hyperplastic and as a result of the loss of colloid they were inactivated. Thyrotropin of the pituitary, therefore, could cause stimulation leading to an increased uptake. When thiourea-treated fish were given thyroxine injections the uptake (45·9 per cent) came to the normal level of controls (45·0 per cent). In the latter, pituitary and thyroxine injections raised the uptake of radiophosphorus from 45·0 to 58·3 and 66·2 per cent respectively. The results show that thyroid has positive influence over the mineral metabolism in fish, but it cannot be said definitely at present whether this control is direct or is indirect through some intermediate agency. Further work is in progress and the detailed report of this work will be published elsewhere.

I am grateful to Prof. F. R. Hayes for his suggestions and encouragement. Thanks are also due to the National Research Council of Canada for the award of a post-doctorate fellowship.

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BIOLOGY

Interspecific Differences in Strontium Uptake by Pasture Plants as a Function of Root Cation-Exchange Capacity

VOSE and KOONTZ[1] have reported differences in strontium contents of pasture plants. As there is evidence that the uptake of cations may be influenced by the root cation-exchange capacity of plants[2–4] it was of interest to compare the observed variation in strontium uptake among the plants with the corresponding root cation-exchange capacities.

Root cation-exchange capacity measurements of several of the plants used by Vose and Koontz[1] had previously been made by a method based on that of Drake *et al.*[4,5] and these results are shown in Table 1. It must be stressed that these results have only limited application as root cation-exchange capacity has been shown to vary with different levels of nutrition[6]. However, when the level of nutrition is similar for all plants (as was the case in the experiments of Vose and Koontz), then the plants should show much the same relative differences in root cation-exchange capacity. Varietal differences in exchange capacity between the plants tested in New Zealand and those used by Vose and Koontz may also cause some perturbation of the results.

By applying Donnan distribution laws to the root membrane–soil solution–soil colloid system, it may be predicted that the higher the cation-exchange capacity of the root surface, the greater will be the proportion of divalent cations within the root micellar solution[7]. As this initial adsorption on the root surface is the first stage of nutrient absorption by plants and, in dilute solutions, appears to have a considerable effect on the quantity and ratio of ions

Table 1

Plant	Root cation-exchange capacity (m.equiv./100 gm. oven-dry roots)
Bromus inermis	25
Lolium perenne	20
Phleum pratense	23
Phalaris tuberosa	18
Dactylis glomerata	23
Medicago sativa	48
Trifolium repens	42
T. pratense	46
Lotus corniculatus	36

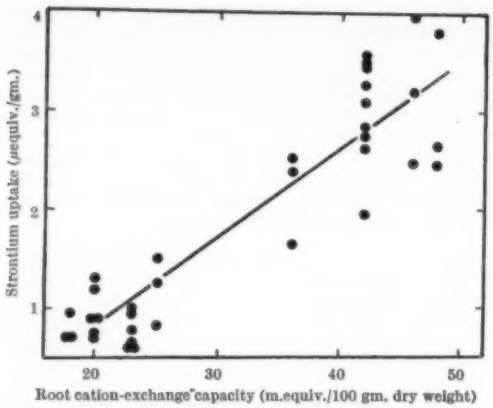

Fig. 1. Strontium uptake (from Vose and Koontz (ref. 1)) as a function of root cation-exchange capacity: $y = 0{\cdot}091x - 1{\cdot}01$; $r = 0{\cdot}91$

absorbed, it would be expected that the uptake of strontium would increase with increase in root cation-exchange capacity. The highly significant correlation between the uptake of strontium recorded by Vose and Koontz[1] and the root cation-exchange capacity (Fig. 1) indicates that the predicted effect of root cation-exchange capacity differences on ion uptake may be a strong factor in determining interspecific differences in uptake of strontium.

The exchange capacity figures used here differ from those obtained by Drake *et al.*[5]. However, the results of these workers significantly correlate with the strontium uptake in a similar manner to those shown here.

While the strontium content varied greatly among species, the calcium to strontium ratio remained constant[1]. This lack of discrimination between the two divalent ions by all species is to be expected if it is accepted that exchange reactions on the root surface determine the ratio of ions absorbed by the plants.

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A Possible Mechanism of Cell-Wall Formation

Goldacre[1] has shown that if a surface film of a lipo-protein on water is compressed, it tends to form folds and ultimately cigar-shaped fibres consisting of a double lipo-protein layer with water inside and outside. It was suggested that such fibres could have been a reaction-space for the original formation of living cells. Danielli[2] formed double lipo-protein layers by dropping dense protein solution through an oil layer into a less-dense protein solution. In this case, the film was spherical in shape and included a layer of oil. Sigwart and Nassenstein[3] have formed spherical shells of water in benzene by ejecting benzene drops from a nozzle preferentially wetted by water.

I have observed an alternative method of formation of a spherical shell of oil stabilized with protein and separating two aqueous phases. A dilute (0·1 per cent) aqueous solution of bovine plasma albumin is placed in a beaker beneath a layer of petroleum ether (80–100° C.); air is then bubbled into the liquid for a few seconds. Spherical shells of the type formed by Danielli[2] may be observed afterwards in the water. Their sizes range up to 2–3 mm. Most of the shells rise to the oil–water interface, but some disintegrate, leaving one small oil droplet.

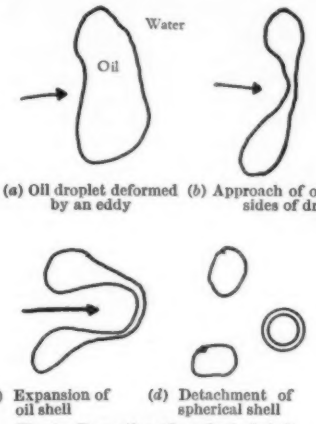
Water
Oil
(a) Oil droplet deformed by an eddy
(b) Approach of opposite sides of droplet
(c) Expansion of oil shell
(d) Detachment of spherical shell

Fig. 1. Formation of a spherical shell

The mechanism of formation of the spherical shell is believed to be the deformation of an oil droplet by an eddy in the way illustrated on Fig. 1. Spherical shells containing air ('inverse bubbles') have been formed in a similar manner[4]. This mechanism of formation of protein-stabilized lipid shells seems to be at least as likely in Nature as those suggested by earlier workers[1,2]. Given a 'primeval soup' of the right composition, the only physical requirement for the mechanism is turbulence.

Thanks are due to the Department of Scientific and Industrial Research for financial assistance while this work was carried out.

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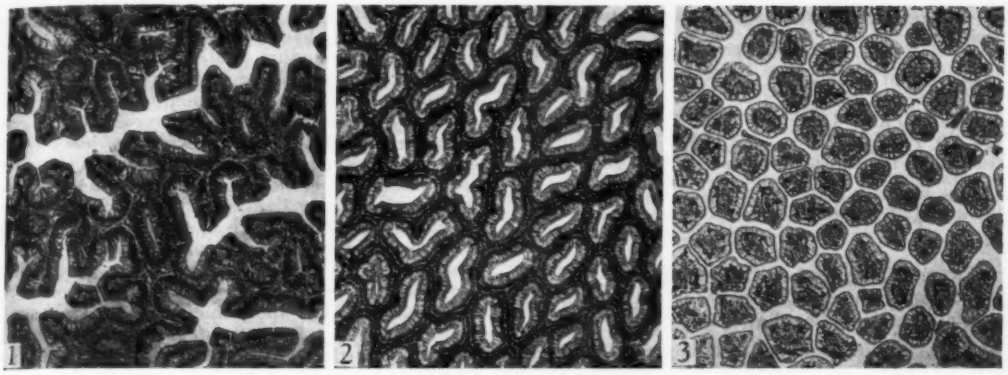
The Intestine in Members of the Genus *Raja* and Host-specificity in the Tetraphyllidea

It was recently found that some species of the genus *Raja* could be distinguished solely on differences in the morphology of their spiral valves, in particular, from the form of their mucosæ. A brief account of the latter is given here because, so far as I know, these differences have not been described previously. In addition, it is thought that variations of this kind may



Figs. 1–3. Facial sections near the surface of the mucosa and from the middle region of the spiral valve of *Raja*. (1) *Raja montagui*. (2) *Raja clavata*. (3) *Raja naevus*

explain, in part, the high degree of host specificity found in the Tetraphyllidea, and may have a bearing on specificity in other orders of the Cestoda. A more detailed account of the work will be published elsewhere.

A facial section near the surface of the mucosa of *Raja montagui* shows (Fig. 1) that villi are absent. Instead the surface is composed of variously arranged and folded ridges which join to form a complicated network. The spaces between the ridges become progressively shallower and less in volume from the anterior to the posterior region of the intestine. Furthermore, these ridges are not always at the same level and therefore the surface of the mucosa has a corrugated and an irregular appearance. In *Raja clavata*, however, the surface is relatively smooth but composed of a fine regular network of ridges (Fig. 2), the intervening spaces leading into tubular crypts which are deeper in the anterior region. *Raja naevus* can be distinguished from these two species because well-developed villi are present (Fig. 3). *Raja brachyura* possesses a mucosa which at first glance is like that of *Raja montagui*, but the ridges are not identical in their detailed structure. The mucosa of *Raja micro-ocellata* resembles that of *Raja clavata*, but a noticeable difference occurs in the form of the muscular layers in the walls of their intestines.

The above differences may be of value, because it is not always easy to identify species of the genus *Raja* on other characters. Parker[1] described and figured variations in the mucosa of different species and in individuals of the same species of the genus. The specimens of *Raja* that I have examined so far, however, have not revealed a significant variation in different individuals. It is possible, therefore, that Parker was dealing with two species in his account of the mucosa of *Raja montagui*. One of his figures supports this view, as it resembles the mucosa of either the spiral valve of *Raja clavata* or that of *Raja micro-ocellata*. It may have been the former, in view of its abundance at Plymouth and because it can occasionally be confused with *Raja montagui*. On the other hand, the external features of *Raja micro-ocellata* are very distinctive.

The variations found in the intestine of *Raja* are not unlike those previously described in some teleost fishes; and although they suggest that a correlation may occur between feeding habits and the structure of the intestine, the possibility of a direct relationship will require a very careful analysis. Little is known of the feeding habits of rays, but preliminary observations suggest that some species of *Raja* may be more selective in their feeding habits than hitherto believed. If a correlation between food and the structure of the intestine does occur in this genus, it will obviously have a very important bearing on the specificity of their cestode parasites.

In recent work on the Tetraphyllidea of elasmobranchs caught off the British Isles, I have been able to confirm that host-specificity is very well marked in these cestodes. I stated earlier[2] that a correlation occurs between the morphology of the intestine in elasmobranchs and the structure of the scolex of their cestodes. It was then suggested that the structure of the gut was a possible factor contributing towards host-specificity in the Tetraphyllidea, and since so little was known of the morphology of the intestine it was decided to investigate this organ in greater detail. It has been confirmed that many genera and species of the Tetraphyllidea, in particular those found in British rays, show a remarkable adaptation to their environment. It is possible that these species can establish themselves successfully in a particular host only if the scolex is suitable for the habitat. This is supported by the literature on the Tetraphyllidea, which suggests that the main features of the scolex are laid down in an early stage of development of the cestode. In addition, preliminary observations on some species of the genus *Echeneibothrium* indicate that young immature individuals possess a scolex which is almost identical in size and structure with that of the adult.

There is also some evidence that the different genera and species of cestodes which are found in the same species of elasmobranch select particular regions of the intestine, and that in many cases different kinds of scolices show a relationship to the depths of the villi, crypts or reticulations of the mucosa as well as to the volume of their intervening spaces.

The above comments are interesting in view of the remarks made by Shulman[3] and Llewellyn[4] on morphological factors affecting the host-specificity of some helminth parasites. The results so far obtained indicate a similar phenomenon in the Tetraphyllidea, but it is assumed that other factors must operate in stimulating these cestodes to settle where optimum conditions occur for protection, nourishment, growth and reproduction. For example, physiological factors, in particular the carbohydrate, fat and protein metabolism of the Tetraphyllidea, are probably very

important for determining their host and habitat specificity.

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External Sexual Differences and their Significance in *Mormyrus kannume* Forskal.1775

Mormyrus kannume is distributed in the Nile system from the five major lakes of Uganda to the delta, and probably also in the Athi River system in Kenya[1]. The present communication was compiled from observations on specimens from the shallow waters of northern Lake Victoria.

There are external sexual differences in *M. kannume*. The base of the anal fin of the female is inclined at an angle of between 17 and 28° to an arbitrary axis between eye and tail fork, whereas in the male this angle lies between 32 and 33°. In the female or immature male, the anal fin is angular in outline, and the slender rays diverge from the base to the periphery of the fin. The anal fin of the mature male is rounded in outline, and the thickened, tapered rays converge to the periphery from the fin base, and due to the constriction at the periphery, when the fin is spread, a depression appears in the centre. Every immature male has an anal fin similar to that of a female, except for the angle at which it is attached to the body. As the testis ripens, the anal fin becomes modified to the form found in the mature male, and after breeding, the testis regresses and the anal fin gradually assumes its original form.

The significance of the modified anal fin cannot be conclusively demonstrated, since the breeding behaviour of *M. kannume* has not been observed. The simultaneous maturation of the gonad and modification of the anal fin in the male, however, indicate that the anal fin has an important function during breeding. If recently killed male and female are placed side by side with their anal fins spread and in contact at the peripheries, a chamber is formed between their anal fins in the manner illustrated in Fig. 1. If this chamber is used to surround the eggs during fertilization, fewer spermatazoa might need to be produced than if the gametes were dissipated into the surrounding water before fertilization. Therefore the use of a fertilization chamber in *M. kannume* during spawning might account for the relatively minute testis of the mature male. In every mature male fish with a total weight of less than 600 gm., the testis never weighed more than 3 gm., and a specimen weighing more than 4 kgm. had a ripe testis which weighed only 7 gm. In a variety of experiments in which macerated testis material and eggs taken from ovulated ovaries were mixed in small volumes of water, effective fertilization was never more than 25 per cent, and frequently less than 10 per cent. This may indicate that the gametes of this species are viable for only a limited period after contact with water, and that fertilization is possible only where immediate intimacy between ovum and sperm occurs. Immediate contact between ovum and sperm may be possible within the fertilization chamber.

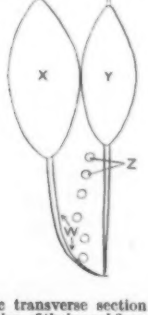

Fig. 1. Diagrammatic transverse section of a pair of fish in lateral contact in the region of their anal fins. (× ½). *W*, Depression in centre of male anal fin; *X*, mature male (34 cm.); *Y*, female (33 cm.); *Z*, extruded ova within the fertilization chamber

M. kannume is a very active gregarious species which probably has the co-ordinative ability to form a fertilization chamber. I have observed that, after being frightened, individuals of *Marcusenius nigricans*, another very active gregarious Mormyrid, form an unusually compact shoal which swims as a single unit through rocky crevices. Individuals of *M. kannume* may also be able to maintain close contact with each other while swimming, and as an extension of this type of behaviour, pairs may be able to form a fertilization chamber during spawning.

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Isolation of Liver Nuclei

A method of isolating pure nuclei from rat liver has been elaborated from those described by Alfrey[1], Schneider and Hogeboom[2], Chauveau[3] and Zbarskij[4].

A rat was fasted for 24 hr. and then killed by decapitation. The liver was perfused with 0·25 *M* saccharose containing 0·0018 *M* calcium chloride and homogenized in a Teflon Potter–Elvehjem homogenizer for 2–3 min. at 1,000 r.p.m. in 0·25 *M* saccharose (+ 0·0018 *M* calcium chloride) at 1:10 dilution. The homogenate was filtered through four sheets of cotton gauze or through Jena *G O* glass filter. The filtrate was afterwards centrifuged for 5 min. at 500 r.p.m. in the horizontal head at 0–2° C. Thereafter we pipetted out the supernatant, which was not sharply separated from the precipitate, and put it on the surface of 0·34 *M* saccharose (+ 0·0018 *M* calcium chloride) and then centrifuged for 10 min. at 2,000 r.p.m. and 0–2° C. in the horizontal head. The supernatant was rejected and the precipitate, composed mainly of nuclei, was resuspended in 0·25 saccharose (+ 0·0018 *M* calcium chloride) and again put on the surface of 2·2 *M* saccharose, the density of which was exactly 1·272 gm./ml. This density, according to Zbarskij[4], allows the sedimentation of pure nuclei only, and at the same time it prevents the sedimentation of smaller cellular structures and of whole cells.

The distance from phase border, that is, from the boundary layer, of 2·2 *M* saccharose and 0·25 *M* saccharose with suspended nuclei to the bottom of tube was 1·5 cm., and the time of centrifuging was 30 min. at 30,000*g* (as calculated for the bottom of tube). The diameter of the tube was 16 mm. The last centrifuging was performed on a super-speed attachment of an MSE refrigerator centrifuge at 0–2° C. The nuclear fraction appears as a light yellow precipitate. When this method is used special attention must be paid in order to attain suitable thickness of the layer of 2·2 *M* saccharose solution and also to the diameter of the tube.

The smear of the nuclear fraction suspended in 0·25 *M* saccharose showed no whole cells, and only few very small fragments of cells were observed. The appearance of the nuclei was like this in living cells. The nuclei have clearly visible nucleoli and normally distributed intranuclear constituents; this is evidence of their normal status. We did not observe any deformation or agglutination of nuclei prepared by this method.

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Case of Apparent Resistance of *Rattus norvegicus* Berkenhout to Anticoagulant Poisons

Over a period of two months, a heavy rat population on a farm in the west of Scotland was treated with two anticoagulant poisons, 'Diphacinone' and 'Warfarin'. Although the treatments were properly conducted, and the rats ate the poison bait freely, little impression was made on the size of the population. There was no evidence of recruitment from outside, and the conclusion drawn was that this population was more than normally resistant to these anticoagulants. Cage tests on 28 animals trapped on the farm to confirm this gave the results shown in Table 1.

Table 1

	Poison	Concentration (per cent)	No. of days feeding	Mortality
Farm rats	'Diphacinone'	0·0025	5	2/9
	'Warfarin'	0·005	5	1/9
	Nil	—	—	0/10
Controls	'Warfarin'	0·005	5	12/15

The remaining 25 rats were further tested at the Ministry of Agriculture, Fisheries and Food laboratories at Tolworth with similar results. More work on the survivors and their progeny is in progress.

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Ophiuroids feeding on Phytoplankton

Recently, Nagabhushanam and Colman[1] have described carrion-eating by some species of ophiuroids including *Ophiocomina nigra* (Abild.) and *Ophiothrix fragilis* (Abild.), from observations both in the laboratory and in the sea, a phenomenon which has been recorded previously[2,3]. The two species mentioned as well as *Ophiopholis aculeata* (L.) have, however, been reported to feed on a wide range of food from seston and plankton to bottom invertebrates[2,3]. In particular, *Ophiothrix fragilis* and *Ophiopholis aculeata* are considered to be mainly suspension feeders[4,5] (using strong ciliary currents[6] in catching detritus), being also facultatively carnivorous. Of the species mentioned, *Ophiocomina* is anatomically well adapted for a carnivorous habit[3]. Nevertheless, all the species mentioned are considered to feed in essentially the same way[2,3].

In order to evaluate the efficiency as suspension feeders of the three species mentioned, we have fed them with phytoplankton labelled with carbon-14. The animals were starved for 1–2 weeks. Two or three individuals of the same species were placed in each of a series of jars containing 400 ml. membrane-filtered sea water to which identical concentrations of carbon-14 labelled plankton algae (natural phytoplankton populations) were added. In every experiment mentioned, constant stirring in 'plunger jars'[7] prevented settling of phytoplankton. The animals were kept in the dark and at a constant temperature.

Two different substrata for the ophiuroids were used; stones and empty shells as one, and the octocoral *Alcyonium digitatum* L. as the other. The latter was used because *Ophiothrix* is often found clinging to it, a phenomenon which is in accordance with the view that *Ophiothrix* is a reophile animal[8]. After 18 hr. the radioactivity of the stomach contents of the ophiuroids was measured.

Table 1. Radioactivity (c./min.) of Stomach Contents of Ophiuroids and Tissue Homogenate of *Alcyonium*

Species	Substratum: Stones and empty shells (c./min.)	Substratum: *Alcyonium digitatum* (c./min.)
Ophiothrix	186	326
	226	120
Ophiopholis	57	5·5
	162	59
	79	20·3
Ophiocomina	0	0
	0	0·9
	0	1·3
Alcyonium	—	110

On the dead substrates, no uptake of phytoplankton by *Ophiocomina* was found. The faint traces of radioactivity measured in specimens of *Ophiocomina* using *Alcyonium* as a substrate is a secondary phenomenon as the octocoral has *a priori* filtered the algae. *Ophiocomina* accordingly cannot be regarded as a suspension feeder, in contrast to the two other species which are extremely efficient suspension feeders, *Ophiothrix* being the superior one.

To measure the rate of removal of plankton algae by starved *Ophiothrix* and *Ophiopholis*, these species were fed with carbon-14 labelled strains of *Sceletonema costatum*. In both species three different concentrations of *Sceletonema* were used (10 : 5 : 1), but the starting activity in the same concentrations was identical. In each of the 2 × 3 jars, three animals were placed in 400 ml. membrane-filtered sea water. The radioactivity in subsamples of 10 ml. water was measured at increasing time-intervals starting with 1 hr.

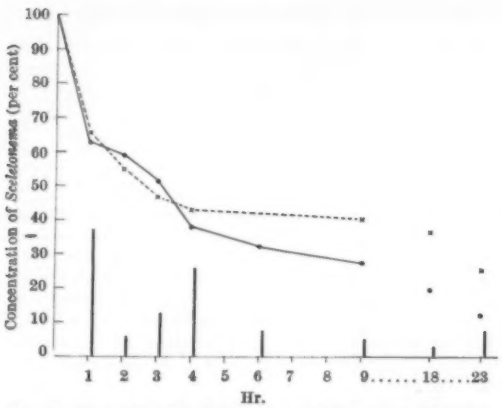

Fig. 1. Curves: Relative decrease in concentration of *Sceletonema* caused by filtration of *Ophiothrix fragilis* and *Ophiopholis aculeata*. ●—● *Ophiothrix fragilis*; ×---×, *Ophiopholis aculeata*. Columns: Relative rate of removal per hr. of *Sceletonema* by *Ophiothrix fragilis*

As the feeding-rates (relative) of the ophiuroids in the three concentrations of algae were almost of the same order of size, the relative average rate of removal of algae is shown in Fig. 1.

Obviously due to hunger, the filtering-rate is always greatest during the first hour and of about the same intensity in both *Ophiothrix* and *Ophiopholis* (respectively 37 per cent and 34 per cent of the algae present were removed). After 4 hr., however, *Ophiothrix* had surpassed *Ophiopholis*, and after 23 hr. *Ophiothrix* had removed 88 per cent of the algae, *Ophiopholis* only 75 per cent.

In all our experiments *Ophiothrix* has proved to be the most efficient filterer of phytoplankton. The highest efficiency was found in three specimens which removed about 50 per cent of the *Sceletonema* present in 400 ml. sea water in 1 hr. This ability probably explains the mass occurrences of this species (> 340 individuals per m.²) in the English Channel[5], where strong tidal currents carry considerable amounts of suspended matter over the food-collecting net of tangled *Ophiothrix* arms. Our demonstration of the absence of filtering in *Ophiocomina nigra* also helps to explain the low ratio of only about 0·6 of this species for every 100 *Ophiothrix fragilis* in the English Channel[5].

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SOIL SCIENCE

Changes with Time in the Availability of Strontium-90 in Soil

Detailed knowledge of the behaviour of strontium-90 in soil is of particular importance in assessing the long-term consequences of its deposition on agricultural land. Experiments have been carried out to determine whether strontium-90 becomes progressively fixed and therefore less available to plants. The majority of the more recent evidence suggests that the availability is decreased to only a small extent, if at all, by changes in the physico-chemical state of strontium-90 in the soil[1]. Deeper penetration through the soil or a more thorough mixing may, however, reduce the quantity of strontium-90 which is absorbed by plants[2].

Further information on this subject is being obtained from a long-term experiment now in progress in this laboratory. The experiment was designed to investigate both the rate at which strontium-90 moves down the soil profile under natural conditions and the manner in which the absorption of strontium-90 by plants changes with time, either as a result of penetration in the soil or because of chemical changes over long periods. Concrete cylinders, 1 yd. wide and 1 yd. deep, were filled with five different soils (Table 1) and left in the open to consolidate for nine months. In the summer of 1956, 1 mc. of carrier-free strontium-90 in aqueous solution was applied to the surface soil of each. Some cylinders were left bare and others were planted with rye grass which was sampled and assayed at regular intervals. In the four seasons for which results are now available, the ratio of strontium-90 to calcium in the grass grown on all soils has decreased by some 20–30 per cent. This reduction could be attributed to either or both of the causes; namely, to slow chemical changes which have reduced the availability of strontium-90 in the soil or to the increasing penetration of strontium-90 down the soil profile. The work here described was undertaken to obtain more direct information on possible changes with time in the physico-chemical form of strontium-90 in the soil; the manner in which it is redistributed down the soil profile will be described elsewhere.

Table 1. Data on Soils

Soil	*p*H	Total exchange capacity m.equiv./100 gm.	Exchangeable calcium m.equiv./100 gm.
I	6·4	10·2	5·1
II	6·7	10·4	6·3
III	5·7	12·2	6·6
IV	7·6	26·2	23·1
V	7·8	28·7	28·0

It is known that chemical extraction procedures do not necessarily give adequate information on the relative availability to plants of strontium and calcium in the soil; difficulties arise owing to the fact that the chemical composition of the soil is to a greater or lesser extent modified by such treatments. An improved procedure which depends on measuring the relative concentration (or potential) of the two ions in a solution with which the soil is in true equilibrium was devised by Schofield and preliminary results have been published[3]. The same principle can be used to compare the availability of strontium ions which have been present in a soil for different periods of time. Dilute calcium chloride solution (0·01 *M*) has been found a suitable medium in which to equilibrate

soil for this purpose; changes in the availability of strontium can be inferred from the relative extent to which material which has been present in the soil for different periods passes into this solution.

This method has been used to compare the availability of the strontium-90 which had been incorporated in the soils for 3½ years, from the cylinders referred to above, with that of strontium-89 which was freshly added to the soil. Small aliquots of air-dry soil from the experimental cylinders were moistened with water or with a solution of carrier-free strontium-89. Seven days later they were shaken for 24 hr. with 0·01 *M* calcium chloride solution (soil/solution ratio of 1 : 8); three replicates of each treatment were employed. After centrifuging, the clear supernatant solutions were decanted and the quantities of strontium-89 or -90 which had passed into solution were determined. The soils were then boiled with 8 *M* nitric acid for 5 min. Repeated extractions with hot concentrated acid were carried out on some samples. The residual activity in the soil was determined with a calibrated beta scintillation counter; it was ascertained that the strontium-89 extracted from the soil, together with that estimated as remaining therein, did not differ significantly from that originally added.

The results in Table 2 show that the amount of freshly added strontium-89 which became equilibrated with the calcium chloride solution ranged from 55·5 per cent in soil I to 26·5 per cent in soil IV. The corresponding values for strontium-90, which had been in the soil for 3½ years, were some 10–3 per cent lower. This difference was significant ($P > 0·01$) for all soils except soil V. The nitric acid extraction removed almost all the strontium-89 from the soils but about 1 per cent of the strontium-90 remained. Even after repeated extractions with concentrated acid, the amount of strontium-90 remaining in the soil was not greatly reduced.

Table 2. The Extraction of Freshly Added Strontium-89, or Strontium-90 which had been present for 3½ Years, from Various Soils

Soil	I	I+Ca*	II	III	IV	V
% removed from the soil by equilibration with 0·01 *M* calcium chloride solution						
^{89}Sr	55·5	46·3	49·1	49·9	26·5	27·9
^{90}Sr	50·7	41·7	44·7	45·6	24·5	27·2
$^{90}Sr/^{89}Sr$	0·91	0·90	0·91	0·91	0·92	0·97
% which remained in the soil after boiling for 5 min. with 8 *M* nitric acid						
^{89}Sr	0·05	—	0·06	0·10	0·05	0·30
^{90}Sr	1·6	1·4	1·0	1·8	1·5	0·9
$^{90}Sr/^{89}Sr$	32	—	17	18	30	3
% which remained in the soil after repeated extractions with nitric acid						
^{89}Sr	0·03	—	0·01	0·03	0·03	0·01
^{90}Sr	1·2	1·0	0·5	1·1	0·6	0·3
$^{90}Sr/^{89}Sr$	40	—	50	37	20	30

* Lime (at the rate of 10 tons/acre) was applied to the soil surface 1 year after the strontium-90.

The result of equilibrating the soils with dilute calcium chloride solution suggests that the availability of the strontium-90 has been reduced by a small amount over the period of 3½ years. Further evidence that some strontium is slowly converted into less-accessible forms is provided by the fact that rigorous extraction with acid displaced less of the strontium-90 which had been present for 3½ years than of the recently added strontium-89.

The mechanism whereby tracer amounts of strontium become firmly bound in soils is of theoretical interest, although these results suggest that the effect of such physico-chemical changes on the absorption of strontium-90 by plants is too small to be of great significance in the assessment of the passage of strontium-90 through food chains. The present experiment will be repeated after the strontium-90 has been in the soils for a longer period.

I am indebted to the late Dr. R. K. Schofield for his advice during the progress of this work.

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VIROLOGY

Mechanical Transmission of the Infectious Variegation Virus of Citrus

Infectious variegation was described as a possible virus disease of citrus in 1931 by Petri[1]. His description of symptoms and his illustrations are similar to those reported by Fawcett and Klotz[2], who transmitted the causal agent by bud-grafting from variegated lemon to sour orange. They noticed that some of the symptoms were similar to those associated with psorosis and suggested that the diseases might possibly be related. Klotz and Fawcett[3] noted that crinkly leaf of lemon is sometimes associated with infectious variegation. Fawcett and Bitancourt[4] were uncertain whether infectious variegation differed sufficiently from crinkly leaf to be considered a separate disease. From cross-protection studies, Wallace[5] concluded that infectious variegation is caused by a strain of the psorosis virus. Grant and Smith[6] reported infectious variegation in Florida and transmitted the causal agent by bud and leaf-piece grafts to several different species of citrus.

Many unsuccessful attempts have been made to transmit mechanically the viruses responsible for tristeza, psorosis, xyloporosis, and exocortis. To date these failures have been explained on the basis of insufficient virus concentration in the inocula, inhibitors, intrinsic properties of the virus, or the susceptibility of the test plant. In 1959, Holmes[7] reported the mechanical transmission of potato mottle virus to three species of citrus. The virus induced only one local lesion on one plant of each species, and was recovered to plants of *Nicotiana tabacum*. The virus was not recovered from non-inoculated leaves of citrus. Holmes[7] concluded that failures to transmit citrus viruses mechanically may have been due to the use of insusceptible or insufficiently susceptible test plants rather than to the presence of inhibitors in citrus foliage.

In the present investigations, leaves of Eureka lemon plants with typical symptoms of infectious variegation—such as vein banding, veinlet banding, speckle, blotch, and crinkle—were frozen (−10° to −20° C.) in a mortar with 1 ml. of 20 per cent sucrose

per gm. of leaf material and with a small amount of activated charcoal. The material was thawed and triturated with a mortar and pestle. The pulp was squeezed through cheese-cloth, and expressed juice obtained in this way was used for subsequent inoculations. Leaves to be inoculated were dusted with 500-mesh 'Carborundum' and inoculated by rubbing with a gauze pad saturated with inoculum or by brushing with a nylon-bristle brush that had been dipped in the inoculum. Inoculated leaves were rinsed with tap water immediately after inoculation. The tests were conducted during January–March. The test plants were either very young seedlings (2- to 4-leaf stage) or older seedlings approximately 12–14 in. high. Controls were comparable test plants that were treated as described except that leaves from healthy Eureka lemon plants provided the source of 'inoculum'.

Table 1. Numbers of Citrus Seedlings infected by Infectious Variegation Virus mechanically transmitted from Eureka Lemon

Exp.	Test species	Relative age of test plants	Transmission Test plants*	Controls
1	Eureka lemon	Young†	7/7	0/7
2	Eureka lemon	Young	5/5	0/5
3	Sour orange	Young	5/5	0/5
4	Eureka lemon	Old‡	3/3	0/3
5	Eureka lemon	Old	0/3	0/3
6	Sour orange	Old	1/3	0/3

* Numerator is number of plants infected after a five-week period and the denominator the number inoculated.
† Seedlings in 2- to 4-leaf stage.
‡ Seedlings 12–14 in. tall.

In three experiments in which the test plants were young when mechanically inoculated, all inoculated plants became infected (Table 1). Some transmission to older plants was obtained, but the results were erratic. Fifteen to twenty-five days after inoculation, some Eureka lemon plants exhibited systemic symptoms identical with those on the source plant. The sour-orange seedlings exhibited vein banding and, later, chlorotic blotches in young leaves, and leaf crinkle and cupping. Some inoculated leaves of Eureka lemon 8–10 days after inoculation exhibited slight chlorotic blotches that afterwards enlarged. When such leaves were cleared in alcohol and stained with iodine, they showed typical starch lesions[8] (Fig. 1).

Both inoculated and non-inoculated leaves of the test plants were examined for virus in two ways. Mechanical inoculations were attempted by the methods described, and leaf pieces were grafted to lemon and sour-orange seedlings. Virus was recovered from young systemically infected leaves as well as from inoculated leaves of experimentally infected plants by leaf piece-graft inoculations. Virus was transmitted by mechanical means from young systemically infected leaves but not from inoculated leaves. The failure to recover the virus by mechanical means from the inoculated leaves may be due to the age of leaf or to low concentration of virus. Old leaves are difficult to triturate with a mortar and pestle, and the virus concentration may be low because of the type of local virus multiplication.

It seems unlikely that use of insufficiently susceptible test plants could account for the previous failures of mechanical transmission of citrus viruses. The results reported here show that the failure of mechanical transmission of at least one citrus virus is not due to low concentration or intrinsic nature of the virus. Earlier attempts may have failed because inhibitors were not removed.

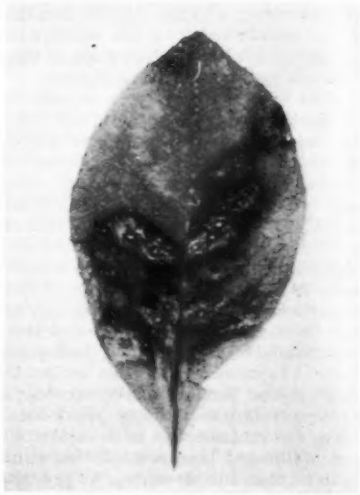

Fig. 1. Leaf of Eureka lemon 14 days after mechanical inoculation with infectious variegation virus, showing expansion of initial local lesions and starch-retention pattern. Leaf was cleared in alcohol and stained with iodine

The effect of sucrose and activated charcoal on the successful transmission of infectious variegation from citrus to citrus is unknown. Possibly enzyme-inhibiting properties of sucrose are somehow related to a mechanism by which sucrose might negate the effects of viral inhibitors in juice of citrus leaves. Moreover, viral inhibitors may be adsorbed by the activated charcoal, thus reducing their interference with infection and multiplication.

The beneficial effects of sucrose and activated charcoal may be elucidated after further study. The important fact at present is that a citrus virus has for the first time been transmitted mechanically from citrus to citrus. Mechanical transmission of any citrus virus immediately facilitates work on the properties of the virus, host-range, test plants, symptomatology, purification, serology, indexing methods, and eventually morphology of the virus particle associated with the disease. Further work on mechanical transmission of variegation and other citrus viruses from the point of view of inhibitors, hosts, and virus concentration is in progress.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 7

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. S. Vajda: "The Building of Models and the Theory of Games".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Impact of Television on Society" opened by Lord James of Rusholme.

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Miss M. V. Griffith: "The Heat Pump in Great Britain".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. W. Idris Jones: "The Future of Chemicals from Coal".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the WOOLWICH POLYTECHNIC SCIENTIFIC SOCIETY, at the Woolwich Polytechnic, Thomas Street, London, S.E.18), at 7 p.m.—Dr. W. Davies: "Some Aspects of the Chemistry of Cancer".

ROYAL GEOGRAPHICAL SOCIETY (joint meeting with the BRITISH SCHOOL OF ARCHÆOLOGY IN IRAQ, at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Prof. M. E. L. Mallowan: "Discoveries at Ur".

Monday, November 7—Tuesday, November 22

ROYAL PHOTOGRAPHIC SOCIETY OF GREAT BRITAIN, MEDICAL GROUP (at 16 Princes Gate, London, S.W.7)—Exhibition of Medical Photography.

Tuesday, November 8

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. B. C. Brookes: "A Philosophy of Applied Science".*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 4.30 p.m.—Dr. A. E. W. Austen, Mr. B. E. Knight, Dr. W. T. Lyn and Dr. T. Priede: "Fuel Injection, Combustion and Noise".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. W. D. Armstrong and Mr. R. M. Wood: "The Dynamic Response of a Distillation Column".

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in Committee Room 11, House of Commons, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Recent Developments and Problems Connected with Supersonic Flight" initiated by Mr. P. A. Hufton and Dr. A. E. Russell, C.B.E.

UNIVERSITY OF LONDON (at Senate House, London, W.C.1), at 5.30 p.m.—Prof. M. G. Kendall: "New Prospects in Economic Analysis" (Stamp Memorial Lecture).*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. W. M. Court Brown: "Chromosome Analysis and Clinical Medicine".* (Sixth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

ILLUMINATING ENGINEERING SOCIETY (at the Federation of British Industries, Tothill Street, London, S.W.1), at 6 p.m.—Mr. F. P. Bentham: "25 Years of Stage Lighting".

INSTITUTION OF THE RUBBER INDUSTRY (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. P. B. Lindley: "Rubber in Engineering". 7 p.m.—Dr. A. N. Gent and Mr. M. Braden: "Mechanics of Attack of Rubber by Ozone".

ROYAL SOCIETY OF MEDICINE, PSYCHIATRY SECTION (at 1 Wimpole Street, London, W.1), at 8 p.m.—Discussion on "The Use of Psychiatric Skills in Industrial Organizations". Opening Speakers: Dr. J. J. O'Dwyer and Dr. Elliott Jaques.

Wednesday, November 9

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 10.30 a.m.—Symposium on "Vertebrate Locomotion".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. P. J. Rendle: "Regulation of Carbohydrate Metabolism by Hormones and Other Factors".*

INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY, COLOUR GROUP (in Lecture Theatre No. 3, Physics Department, Imperial College, London, S.W.7), at 3 p.m.—Mr. L. B. Happé: "Some Problems of Colour Reproduction in the Cinema"; Mr. J. Vickers: "Problems in Printing Colour Negatives"

PALÆONTOLOGICAL ASSOCIATION (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. Dorothy Hill (University of Queensland): "The Fine Structure of the Coral Skeleton and its Application in Classification".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. H. E. Rose: "Vibration Mills and Vibration Milling".* (First of three lectures. Further lectures on November 16 and 23).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Charles Wilcocks: "Aspects of Medical Investigation in Africa—Trypanosomiasis".* (Third of four Heath Clark Lectures. Further lecture on November 10.)

ROYAL INSTITUTE OF CHEMISTRY (at the Royal Institution, 21 Albemarle Street, London, W.1), at 5.45 p.m.—Dr. T. C. Waddington: "Liquid Hydrogen Halides as Ionizing Solvents" (Meldola Medal Lecture).

INSTITUTE OF INFORMATION SCIENTISTS (at the College of Preceptors, 2 Bloomsbury Square, London, W.C.1), at 6.15 p.m.—Mr. C. A. Ronan: "Problems in Transliteration of Cyrillic".*

BRITISH INSTITUTION OF RADIO ENGINEERS, MEDICAL AND BIOLOGICAL ELECTRONICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. T. G. Brown: "Diagnostic Applications of Ultrasonics".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Meeting on "Food Technology in Italy". Speakers: Mr. A. E. Billington, Mr. R. D. Mason, Dr. J. R. Nicholls and Dr. J. Tramer.

SOCIETY FOR PSYCHICAL RESEARCH (at Kensington Town Hall, Kensington High Street, London, W.8), at 7 p.m.—Mr. Christopher Scott: "The Douglas Johnson T.V. Sitting".

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, Westminster, London, S.W.1), at 7 p.m.—Mr. E. C. Cookson and Mr. A. H. Emerson: "Railway Modernisation and Electrification".

Thursday, November 10

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. A. Bogdan: "Grass Pollination by Bees in Kenya"; Dr. J. B. Free: "The Pollination of Fruit Trees by Honeybees".

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. F. C. Williams, F.R.S., Dr. E. R. Laithwaite, Mr. J. F. Eastham and Mr. L. S. Piggott: "The Logmotor—a Cylindrical Brushless Variable-Speed Induction Motor"; Prof. F. C. Williams, F.R.S., Dr. E. R. Laithwaite, Mr. J. F. Eastham and Mr. W. Farrer: "Brushless Variable-Speed Induction Motors using Phase-Shift Control".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. M. R. Pollock: "Physiological Adaptation in Micro-organisms".* (Seventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

OIL AND COLOUR CHEMISTS' ASSOCIATION (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. A. G. North: "Water-Thinnable Stoving Finishes".

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 7.30 p.m.—Prof. R. S. Nyholm, F.R.S.: "Electron Configuration and Structure of Transition-metal Complexes" (Tilden Lecture).

RADAR AND ELECTRONICS ASSOCIATION (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p.m.—Mr. C. A. Burgess: "V.H.F. Aerial Techniques".*

ROYAL INSTITUTE OF CHEMISTRY (at Croydon Technical College, Fairfield, Croydon), at 7.30 p.m.—Dr. H. J. Barber: "Some Aspects of Chemotherapy".

Thursday, November 10—Friday, November 11

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m. daily—Discussion on "Progress and Needs of Marine Science" led by Dr. G. E. R. Deacon, F.R.S., and Dr. F. S. Russell, F.R.S.)

Friday, November 11

ASSOCIATION OF APPLIED BIOLOGISTS (at the British Museum (Natural History), South Kensington, London, S.W.7), at 10.50 a.m.—Meeting on "Applications of Radiation in Biology".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Royal Aeronautical Society, at Savoy Place, London, W.C.2), at 5.30 p.m.—The Rt. Hon. The Viscount Caldecote, D.S.C.: "The Future of 'Electrics' and 'Electronics' in Aircraft and Guided Missiles".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. J. Harrison: "Experiments with Diving Seals".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or ASSISTANT LECTURER (preferably with experience in chemical or biochemical aspects of pharmacology) IN THE DEPARTMENT OF PHARMACOLOGY—The Registrar, The University, Leeds 2 (November 14).

BIOCHEMIST (honours graduate with some research experience), to assist Dr. R. M. C. Dawson on isotopic studies on lipid metabolism in rumen micro-organisms and in the ruminant digestive tract—The Secretary, Institute of Animal Physiology, Babraham, Cambridge (November 15).

FISHERIES ADVISER (with a university degree in biology and a minimum of five years experience on both research and development) in the Ministry of Animal Resources, Sudan, for duties which will include the biological and technological investigations leading to the establishment of a profitable inland fisheries industry, and also the training of Sudanese assistants—The Appointments Secretary, Sudan Embassy, 3 Cleveland Row, St. James's, London, S.W.1 (November 15).

LECTURER IN THEORETICAL PHYSICS—Prof. A. Salam, Physics Department, Imperial College of Science and Technology, London, S.W.7 (November 15).

ASSISTANT EXPERIMENTAL OFFICER (with a degree or equivalent qualifications in chemistry) IN THE DEPARTMENT OF PEDOLOGY, for duties mainly concerned with the chemical analysis of peat and herbage—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (November 19).

RESEARCH ASSISTANT (graduate in medicine, and preferably some physiological knowledge and experience in simpler biochemical estimations) IN THE DEPARTMENT OF ANÆSTHETICS—The Registrar, King's College (University of Durham), Newcastle upon Tyne (November 19).

READER (with high academic qualifications and good experience in teaching and research) IN CIVIL ENGINEERING—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11 (November 28).

CHAIR OF PURE MATHEMATICS—The Registrar, University College, Singleton Park, Swansea (November 30).

LECTURER IN EDUCATION at the University of New England, Armidale, N.S.W., Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, November 30).

LECTURER IN THE DEPARTMENT OF STATISTICS, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, November 30).

LIBRARIAN—The Secretary, The Queen's University, Belfast (November 30).

RESEARCH FELLOW IN BOTANY at the University College of Rhodesia and Nyasaland for research into the autecology of *Salvinia auriculata*, to be conducted partly at Lake Kariba and partly at the College—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

SENIOR LECTURER or LECTURER IN CHEMICAL ENGINEERING at the University of Natal, Durban—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, November 30).

SENIOR LECTURER or LECTURER IN MECHANICAL ENGINEERING at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, November 30).

UNIVERSITY LIBRARIAN—The Registrar, The University, Leicester (November 30).

LECTURER (with an honours degree and preferably some operating or research experience in some aspect of mining engineering) IN MINING ENGINEERING in the School of Mining Engineering and Applied Geology, University of New South Wales, Kensington, N.S.W.—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2 (Australia and London, December 2).

LECTURER IN ORGANIC CHEMISTRY at Makerere College (The University College of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 10).

CHAIR OF PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (December 31).

PIG INDUSTRY DEVELOPMENT AUTHORITY FELLOW, for research in pig health—The Secretary, Royal College of Veterinary Surgeons Trust, 9 Red Lion Square, London, W.C.1 (February 25).

ASSISTANT, Grade B (with an honours degree in physics, and research or industrial experience in some branch of applied physics), IN THE DEPARTMENT OF PHYSICS—The Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7.

CHEMIST (with a first- or good second-class honours degree in chemistry or an equivalent qualification, and preferably experience in the inorganic analytical field and a knowledge of geochemical techniques) in Nyasaland, to take charge of the Geological Survey Departments, chemical laboratory, and to undertake assaying and chemical testing of rocks, including full silicate rock analyses—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.105/4/04.

CHEMIST (with an honours degree in chemistry, veterinary/agricultural or medical experience, and some experience in biochemistry) with the Government of Tanganyika, to supervise the Chemical Section of the Veterinary Research Laboratory (duties will include frequent safaris for experiments in the field)—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/8/020.

LECTURER (graduate in physics, preferably with teaching and research experience); and an ASSISTANT LECTURER (preferably with teaching or industrial experience) IN PHYSICS—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

LECTURER IN PSYCHOLOGY—The Principal, West End Hospital Speech Therapy Training School, 59 Portland Place, London, W.1.

OFFICIAL FELLOW AND TUTOR (preferably with interests in pure mathematics) IN MATHEMATICS—The College Secretary, Balliol College, Oxford.

RESEARCH ASSISTANT (with a first- or second-class honours degree in mathematics or science) IN THE SUB-DEPARTMENT OF COMPUTATION, for duties which will include the preparation of programmes for the Ferranti "Pegasus" computer, and the provision of assistance to other users of the machine—The Registrar, The University, Southampton.

TECHNICIAN (with experience of microbiological techniques and preferably some knowledge of biochemical or brewing procedures), to take charge of large-scale growth of micro-organisms by continuous fermentation methods and to be concerned with growth and purification of bacterial viruses—The Deputy Director, Medical Research Council Biophysics Research Unit, King's College, Strand, London, W.C.2.

WHOLE-TIME BIOCHEMIST (non-resident), for hospital biochemical work in the Mansfield Area Pathology Service—The Hospital Secretary, King's Mill Hospital, Sutton-in-Ashfield, Notts.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Agriculture, Fisheries and Food. Report of the Working Party on a Welsh Agricultural College, 1960. Pp. ii+32. (London: H.M. Stationery Office, 1960.) 1*s.* 9*d.* net. [38

British Broadcasting Corporation. B.B.C. Engineering Monograph No. 31: The Power Gain of Multi-Tiered V.H.F. Transmitting Aerials. By P. Knight and G. D. Monteath. Pp. 38. (London: British Broadcasting Corporation, 1960.) 5*s.* [48

Durham University Appointments Board. Second published Annual Report, 1958–1959. Pp. 7. (Durham: The University Appointments Board, 1960.) [48

Bulletin of the British Museum (Natural History). Entomology. Vol. 9, No. 4: A Review of the African Species of the Genus *Chasmatopsyche* (Trichoptera, Hydropsychidae), with special reference to those of Southern Africa, and the Ephemeroptera Types of Species described by A. E. Eaton, R. McLachlan and F. Walker, with particular reference to those in the British Museum (Natural History). By D. E. Kimmins. Pp. 253–318. 20*s.* Vol. 9, No. 5: The *Jamides cuchylas* Complex (Lepidoptera: Lycaenidae). By G. E. Tite. Pp. 319–336+plate 21. 8*s.* Zoology. Vol. 6, No. 7: The Ecology and Taxonomy of some Angola Birds (Based on a collection made in 1957). By B. P. Hall. Pp. 367–453+plates 5 and 6. 40*s.* (London: British Museum (Natural History), 1960.) [48

Other Countries

Canada: Department of Northern Affairs and National Resources. National Museum of Canada. Bulletin No. 162: Contributions to Anthropology, 1957. Pp. iii+203 (28 plates). 2 dollars. Bulletin No. 168: List of the Marine Fishes of Canada. By D. E. McAllister. Pp. ix+76. 1.25 dollars. (Ottawa: Queen's Printer, 1960.) [28

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 41 (June 1960): Recent and Future Mortality Trends. Blood Pressures in Men and Women. Longevity of Industrial Policyholders in 1959. Many Women Killed in Accidents. Pp. 12. (New York: Metropolitan Life Insurance Company, 1960.) [28

University of California Publications in Geological Sciences. Vol. 37, No. 1: Fossil Mammalia from the Early Wasatchian Four Mile Fauna, Eocene of Northwest Colorado. By Malcolm C. McKenna. Pp. 1–130. (Berkeley and Los Angeles: University of California Press: London: Cambridge University Press, 1960.) 2.50 dollars. [28

Proceedings of the California Academy of Sciences. Fourth Series. Vol. 31, No. 1: The Wasps of the Genus *Plenoculus* (Hymenoptera: Sphecidae, Larrinae). By Francis X. Williams. Pp. 1–49. Vol. 31, No. 2: Interpopulation Variation in the Colubrid Snake *Natrix pryeri* from the Riukiu Islands, with description of a New Subspecies. By E. V. Malnate and Harold E. Munsterman. Pp. 51–67. Vol. 31, No. 3: Further Records of Neotropical Tabanidae (Diptera) Mostly from Peru. By Cornelius B. Philip. Pp. 69–102. (San Francisco: California Academy of Sciences, 1960.) [28

Abridged Scientific Publications from the Kodak Research Laboratories, Vol. 34, 1952. Pp. 253+xvi. (Rochester, New York: Eastman Kodak Company, 1960.) [28

Food and Agriculture Organization of the United Nations. World Forest Inventory, 1959. Pp. xii+137. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1960.) 12*s.* 6*d.*; 2.50 dollars. [28

Western Australia. Geological Survey—Mineral Resources of Western Australia. Bulletin No. 7: Iron Ores in Western Australia. Collated by R. R. Connolly. Pp. 103. Bulletin No. 113: Miscellaneous Reports for 1956. Pp. 206+35 plans in separate atlas. (Perth: Government Printer, 1959.) [28

Transactions of the American Philosophical Society. New Series. Vol. 50, Part 6: The Paleocene Pantodonta. By Elwyn L. Simons. Pp. 99. (Philadelphia: American Philosophical Society, 1960.) 2.50 dollars. [28

Atomic Energy of Canada Limited. Annual Report, 1959–60. Pp. 36. (Ottawa: Queen's Printer, 1960.) 25 cents. [38

New Zealand Forest Service: Forest Research Institute. Technical Paper No. 28: A Root and Stem Canker of Exotic Conifers in New Zealand. By J. W. Gilmour. Pp. 19+8 plates. (Wellington: Government Printer, 1959.) [38

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